Degrees of Change:

Varying Patterns of Plasticity Across Warming Climatic Landscapes in a Viviparous Lizard

A thesis submitted in fulfilment of the requirements for the dagree of Doctor of Philosophy

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UNIVERSITY *of* TASMANIA

Statements and Declarations

Declaration of Originality

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Statement of Co-Authorship

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George Cunningham (candidate and primary author) conceived of the study and performed the statistical analysis, performed the field work and was responsible for manuscript presentation. Erik Wapstra (author 3) and Geoffrey While (author 2), contributed to the formalization, development, and refinement of the study, carried out field work and provided feedback during manuscript presentation.

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Cunningham, G.D., Fitzpatrick, L.J., While, G.M. & Wapstra, E. (2018). Plastic rates of development and the effects of thermal extremes on offspring fitness in a viviparous lizard. *Journal of Experimental Zoology*, **329:** 262 - 270.

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We the undersigned agree with the above stated proportion of work undertaken for each of the above published (or submitted) peer-reviewed manuscripts contributing to this thesis:

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ABSTRACT

As climates warm, populations of species will be faced with novel climatic environments, to which they may not be adapted. Due to local adaptation and biogeographic and demographic history, populations are likely to differ in their response to changed conditions in the short-term, and their capacity to evolve in response to changed conditions in the long-term. However, predictions of the effects of climate change often assume that short-term responses will be homogeneous both among populations, and among the individuals within them. Such assessments also often ignore the capacity for these responses to evolve in response to novel selective pressures, assuming that the relationship between environmental temperature and phenotype will be stable, both geographically and temporally. Because reptiles are ectotherms, environmental temperatures determine many aspects of their physiology, behaviour and ecology. As such, reptiles are thought to be at especially high risk from changing climates. Understanding the short-term effects of temperature, how patterns of responses have evolved, and the capacity of populations to adapt into the future has, therefore, never been more important in the light of ongoing climate change.

In this thesis I use the spotted snow skink, *Niveoscincus ocellatus*, as a model organism to investigate climatic drivers of intraspecific patterns of thermal developmental plasticity. Previous research has demonstrated that climatically distinct populations of *N. ocellatus* differ in a host of phenotypic characteristics, including morphology, physiology, behaviour and, intriguingly, sex determination system. I use a range of techniques incorporating a long-term field study, experiments, and simulation modelling to explore the short- and long-term effects of changing climates, focussing on two key traits: phenology and offspring sex.

I demonstrate that thermal reaction norms of phenology are consistent among populations, but that populations differ in the degree of variation at the phenotypic level between the individuals within them. I show that birth date has significant consequences for growth during the first year of life, but does not affect survival. These findings have important consequences for population persistence. As climates warm, birth dates will advance across the range of the *N. ocellatus* and may have positive affects on population persistence.

I also demonstrate intraspecific variation in sex determination systems among populations in *N. ocellatus*. I show that environmental temperatures do not affect offspring sex in a highland population. In a lowland population, however, I show that environmental temperature affects offspring sex, and that this effect is not altered by other variables. Using an individual-based evolutionary simulation model, parameterised with data from a long-term study of two populations, I then extend an explanatory conceptual framework, which explains the evolution of sex determination systems as arising from sex-specific benefits of date of birth, so that it applies across the range of the species' present-day climatic landscape. Finally I use this model to predict how patterns of selection for sex determination systems and sex ratios will be altered throughout the species' range.

My thesis has contributed significantly to our understanding of the climatic and ecological factors that have shaped patterns of variation among and within populations and how species, and the populations within them, will respond to changing climates.

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Chapter 1:

General Introduction

Since the beginning of the 20th century global temperatures have risen by approximately 0.78 °C, and are likely to exceed a rise of 1.5 °C by the end of the 21st century (IPCC, 2014). These increasing temperatures have been shown to have concomitant effects on a number of other climatic variables, including precipitation and wind patterns, ocean circulation, incidences of extreme events and climate variability (Hartmann *et al.*, 2014). Whilst climates have changed throughout history, the scale and rate of current warming are as great as at any time in the last 65 million years (Diffenbaugh & Field, 2013; Kemp *et al.*, 2015). Altered climates are already having significant consequences for species, communities and ecosystems (Walther *et al.*, 2002; Pecl, 2017; Bonebrake *et al.*, 2018). Species across all taxa and geographic regions are responding to warming temperatures by shifting range to track climatic niches (Lenoir & Svenning, 2014). Whilst individual species differ in the magnitude of these distributional shifts, a clear trend has emerged, whereby species' ranges are shifting to higher latitudes and towards higher elevations (Chen *et al.*, 2011; but see Lenoir *et al.*, 2010).

Phenotypic plasticity

Whether populations continue to persist in currently occupied locations, or colonise new areas, they will be faced with novel selective pressures, to which they may not be adapted. One mechanism by which species may persist under altered environmental conditions is phenotypic plasticity: the capacity for a genotype to be expressed as different phenotypes across an environmental gradient (Scheiner, 1993). By allowing individuals to optimise their fitness across environmental contexts, phenotypic plasticity, in cases where it is adaptive, may mitigate the negative consequences of altered climates (Ghalambor *et al.*, 2007). However, the capacity of species to respond via this mechanism may be limited. Indeed, because adaptive phenotypically plastic responses have evolved within a particular environmental context, responses may prove maladaptive in novel or extreme conditions beyond those that they have experienced historically (Ghalambor *et al.*, 2007; Reed *et al.*, 2010; Chevin and Hoffmann, 2017). Thus, realised phenotypes in novel environments may result either

in enhanced fitness, leading to population growth, or reduced fitness, resulting in population reduction or even collapse.

Adaptive phenotypic plasticity, thus constitutes a possible mechanism by which species, and the populations that make them up, may be able to persist in rapidly changing environmental contexts. It is not surprising, therefore, that researchers are increasingly attempting to incorporate phenotypic plasticity into species' risk assessments (Kearney & Porter, 2009; Urban *et al.*, 2016). To do so, however, we require information describing phenotypically plastic characteristics across environments and the consequences of these expressed phenotypes on organismal fitness in the wild. These considerations are complicated, however, because patterns of phenotypic plasticity can be expected to vary within species (e.g., Nussey *et al.* 2007; Beldade *et al.*, 2011; Gomez-Mestre & Jovani, 2013). Where they are adaptive, patterns of phenotypic plasticity are, themselves, evolved responses to environmental conditions. Thus, we should expect these patterns to differ among populations, especially climatically distinct populations, due to local adaptation if populations have different selective optima (Nicotra *et al.*, 2007; Valladares *et al.*, 2014). Despite this, models, even those that attempt to account for phenotypic plasticity, often assume, either implicitly or explicitly, that responses will be homogeneous within species. Furthermore, because patterns of phenotypic plasticity, where they are adaptive, are under selection, they may change over time as altered selective environments drive an evolutionary response and populations (if they survive long enough) become adapted to new conditions (Visser, 2008).

Just as populations can be expected to vary in patterns of phenotypic plasticity, populations can also be expected to vary in their evolutionary responses to changed conditions due to several factors. First, the direction and strength of selection is likely to vary among populations due to environmental differences among locations. Second, the capacity of populations to evolve depends, in part, on the degree of genetic diversity within them. Thus, factors that affect genetic diversity such as biogeographic history, past population bottlenecks, demographics, connectivity with other populations, genetic drift, and founder effects, may also affect populations' evolutionary capacity. Third, local adaptation is limited by the degree to which populations are genetically isolated from other populations (García-Ramos & Kirkpatrick, 1997; Kawecki & Ebert, 2004). Lastly, phenotypic plasticity, which, as

described above, may vary among populations, can act to either promote evolution by enabling populations to persist long enough for populations to evolve, or hinder adaptation by reducing selective pressures (Chevin & Lande, 2010; Chevin, 2013). Indeed, where they are adaptive, patterns of plasticity will, themselves, evolve over time. If we are to accurately predict the effects of changing climates on species persistence it is critically important that we understand how patterns of phenotypic plasticity should be expected to vary among and within populations, the selective pressures that have driven selection for observed patterns, and the capacity of populations to evolve (Kearney & Porter, 2009; Hoffmann & Sgró, 2011). These considerations are especially important in taxa, such as ectotherms, in which temperature has strong effects and for traits, such as phenology and offspring sex ratios, that determine population persistence and growth.

Phenology as a plastic trait

One suite of traits that can have substantial effects on population persistence and growth is an alteration of the timing of life history events (i.e., phenology). Altered phenologies, for instance the timing of migration (e.g., Hüppop & Hüppop, 2003; Sparks *et al.*, 2005), flowering (e.g., Fitter & Fitter, 2002), laying (e.g., Crick *et al.*, 1997) or spawning (e.g., Beebee, 1995), birth or hatching (e.g., Visser & Holleman, 2001; Husby *et al.*, 2010) and the length of growing seasons (e.g., Menzel & Fabian, 1999) have been reported across trophic levels and a broad range of taxa, (e.g., Permesan & Yohe, 2003; Root *et al.*, 2003; Menzel *et al.*, 2006; Parmesan, 2007; Ge *et al.*, 2016; Cohen *et al.*, 2018, Post *et al.*, 2018), including ectotherms (e.g., While & Uller, 2014; Noble *et al.*, 2018). The effects of altered phenologies on population persistence may be either positive or negative. For example, early emergence from hibernation, extended active seasons and earlier birth can result in an increased amount of time available for growth and condition building (McCaffery & Maxwell, 2010; Phillimore *et al.*, 2012). On the other hand, because species do not exist in isolation, shifts in phenology may lead to alterations in biotic interactions, such as those between predators or prey. Where the phenologies of animals and their food sources are affected to differing extents, times of high nutritional requirements (e.g., prior to ovulation, during pregnancy or during parental provisioning of offspring) may

not coincide with the availability of these resources. Such "phenological mismatches" may lead to population decline or even collapse (Visser & Holleman, 2001; Durant *et* al., 2007; Both *et al.*, 2009; Thackeray *et al*., 2016; Zhang *et al.*, 2017; Noble *et al.*, 2018). Similarly, if phenologies of species with similar niches converge as the result of altered conditions, then populations may face increased competition for resources (Lancaster *et al*., 2016).

Reptiles at risk

Concerns about the effects of altered climates on species are especially pertinent for reptiles. As ectotherms, reptile physiology and, consequently, many aspects of their behaviour, ecology and development, are strongly affected by environmental temperature (Cowles & Bogart, 1944). For example, the thermal environment that developing offspring face can have significant consequences on a host of phenotypic characteristics at birth and later in life. These include such factors as developmental rate (and therefore, hatching or parturition dates), physiology, morphology, performance and survival (Noble *et al.*, 2018). Because of these factors, reptiles are considered to be at especially high risk from changing climates (Tuberville *et al.*, 2015; Böhm *et al.*, 2016). Indeed, it has been estimated that, amongst reptile populations, 4% have become extinct worldwide since 1975 and that 39% of local populations and 20% of species may become extinct by 2080 (Sinervo *et al.*, 2010). Many reptiles have highly specific requirements for metabolism and reproduction and often have narrower distributional ranges (Anderson, 1984; Anderson and Marcus, 1992) and lower dispersal rates (e.g., Olsson & Shine, 2002; Araújo *et al.,* 2006; Escobedo-Galvan *et al.*, 2011) than other terrestrial vertebrates, increasing the susceptibility of reptilian species, and populations to extinction. Furthermore, some reptile species are thought to be at especially high risk because of the sex of offspring is intimately associated with climate.

Sex determination in reptiles

Among reptiles there are two primary sex determination mechanisms. In many species of reptiles, as in mammals and birds, sex is determined, at conception, by

genes (genetic sex determination, GSD). In other species, however, sex is determined after fertilisation by temperature (temperature-sensitive sex determination, TSD). Patterns of sex determination vary among reptile lineages (The Tree of Sex consortium, 2014). In some taxa, sex determination mechanisms appear to be highly conserved. For instance, all known snakes have GSD, whilst crocodiles have TSD. Other groups, however, appear to be surprisingly labile in respect to sex determination. Evolutionary transitions between sex determination systems have occurred multiple times, and alternative patterns have been reported even in closely related species (Sabath *et al.*, 2016).

Janzen (1994) first raised the concern that species with TSD might be at especially high risk from rapidly warming climates (see Böhm *et al.*, 2016). This is because, as climates diverge increasingly from historical patterns to which populations are adapted, offspring sex ratios are likely to become increasingly skewed (Mitchell & Janzen, 2010; Boyle *et al.*, 2014). Theoretical studies have suggested that, if offspring sex ratios translate into skewed ratios in the breeding population, this can lead to rapid population collapse (Janzen, 1994; Le Galliard *et al.*, 2005; Mitchell *et al.*, 2010; Schwanz *et al.,* 2010; Neuwald & Valenzuela, 2011; Boyle *et al.*, 2014). In the absence of sex specific mortality, population growth is generally limited by the number of females rather than males, at least in species, including most reptiles (Uller & Olsson, 2008), that are not monogamous (Wedekind, 2002). Thus, species in which a skew towards males occurs at higher temperatures (e.g., Tuatara: Mitchell *et al.*, 2008) are considered to be at higher risk than those in which a skew towards females occurs at high temperatures, although even these species may be at risk if temperatures rise to the extent that extreme female skews result (Wapstra *et al.*, 2009; Hays *et al.*, 2017). Short-lived species are considered to be at especially high risk, because even a few (or a single) years with extreme temperatures can result in extreme skews in the breeding population (Bull & Bulmer, 1989).

In reptiles with TSD, developmental temperatures affect both offspring sex and developmental rates (and, therefore phenology of parturition dates). As with other thermally plastic traits, we should expect that climatically distinct populations will differ in the extent to which these phenotypes are affected across temperature gradients. To complicate matters further, changes in phenotype resulting from physiological plasticity may be offset by (locally adapted) behavioural plasticity.

Oviparous species, for instance, may buffer developing offspring against the effects of higher temperatures by altering nesting phenology (Schwanz & Janzen, 2008), digging deeper nests, or by choosing cooler, less exposed nesting sites (Doody *et al.*, 2006; but see Telemeco *et al.*, 2009; Schwanz, 2013; Mainwaring *et al.*, 2016). For these species, however, these behavioural decisions must, in general, be made when eggs are laid. In contrast, females in viviparous species may be able to buffer against the effects of temperature throughout the whole of gestation by altering their basking behaviour (Schwarzkopf & Shine, 1991; Schwarzkopf & Andrews, 2012; Shine, 2012). In both oviparous and viviparous species, however, behavioural compensation for higher temperatures may be limited by the fitness consequences of altered behaviour. For instance, altered basking decisions may increase exposure to predators or reduce the time spent foraging. Understanding the climatic and ecological drivers of patterns of phenotypic plasticity in the wild, and how these patterns vary among and within populations requires long-term datasets tracking phenotypes across populations, across multiple years (Nussey *et al.*, 2007). Datasets of this type are, however, rare (but see Coulson *et al.*, 2000; Saether *et al.* 2005), especially in reptile systems.

Thesis overview

In the course of my candidature, I have used a long-term dataset tracking life history traits in populations from the climatic extremes (see Figure 1) of the spotted snow skink, *Niveoscincus ocellatus.* In this thesis I make extensive use of this long-term dataset, combined with manipulative experiments and simulation modelling to investigate between- and within-population variation in the effect of climate on phenotypically plastic traits that are likely to be affected by changing climates. *Niveoscincus ocellatus* is a small viviparous skink (3-10 g, 60-75 mm snout-vent length (SVL)), endemic to Tasmania, Australia. The species is a trophic generalist (Wapstra & Swain, 1996), and inhabits areas of rocky scree across a wide altitudinal range from sea level to 1200 m (Uller *et al.*, 2011; Cadby *et al.*, 2014). After reaching maturity, females give birth to a single litter each year. Consequently populations are found across a broad range of climates (Figure 1) from comparatively warm benign coastal areas to cold subalpine/alpine areas. Across this range, there is evidence of

local adaptation (Cadby *et al.*, 2014), and evidence of among-population genetic isolation and differentiation (Cliff *et al*, 2015; Hill *et al.*, 2018).

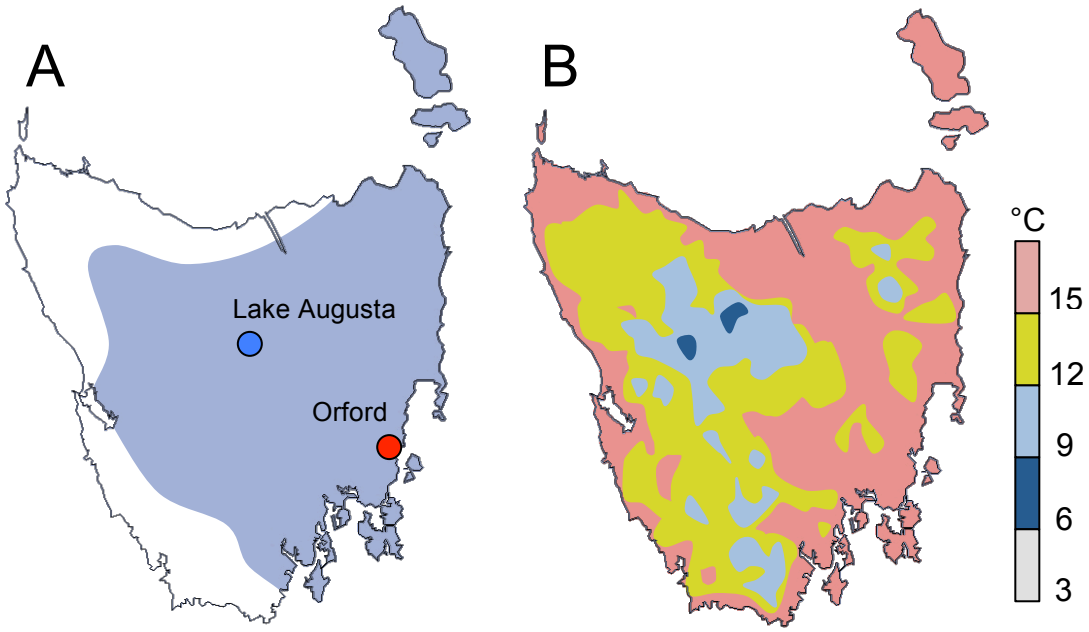

Figure 1: Map of Tasmania showing A) the approximate distribution (shaded area) of *Niveoscincus ocellatus* and B) The mean (1961-1990) annual maximum temperature across Tasmania. Circles show the locations of our two long-term study sites: a highland site at Lake Augusta (blue), and a lowland site at Orford (red).

The highland site is located at Lake Augusta on Tasmania's Central Plateau (41°86' S, 147°53' E; elevation 1150 m) whilst the lowland site is located near Orford on Tasmania's east coast (42°55' S, 147°87' E; elevation 30 m). Because of their differences in elevation and location, these sites have substantially different climates (Figures 1 & 2). The lowland site is characterised by relatively warmer temperatures than the highland site, and temperatures vary little between years. In contrast, the highland site experiences lower mean temperatures, which vary substantially from year to year (Luh *et al.*, 2015).

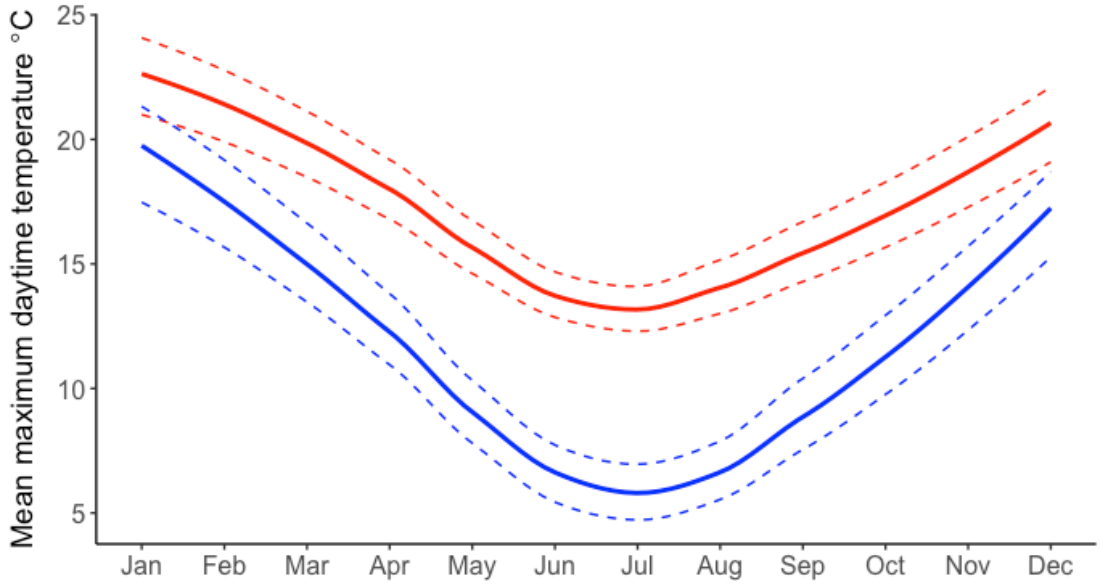

Figure 2: Long-term mean of mean monthly maximum daytime temperature at the lowland (red) and highland (blue) long-term study sites. Dashed lines show 10th and 90th decile range. Data from Australian Bureau of Meteorology website.

Previous research on these populations, including research based on the long-term dataset, has demonstrated that these populations differ in a number of physiological and behavioural characteristics. In the highland population, females reach maturity later and at a larger size (Wapstra *et al.*, 2001, Pen *et al.*, 2010), produce larger offspring (Wapstra & Swain, 2001), have larger litters (Wapstra & Swain, 2001; Pen *et al.*, 2010), ovulate later and give birth later (Wapstra *et al.* 1999), give birth over a shorter period (Wapstra *et al.*, 1999), bask more intensely (Uller *et al.*, 2011; Caldwell *et al.*, 2017), have higher preferred body temperature (Cadby *et al.*, 2014), and have a shorter active season (Wapstra & Swain, 1996, 2001; Luh *et al.*, 2015) than those in at the lowland site. Furthermore, temperature has a different effect on offspring sex in these populations. At the lowland site, offspring sex ratios are skewed, relative to the long-term sex ratio, towards females in warm years, and towards males in cooler years. In contrast, at the highland site offspring sex ratios are not affected by temperature and vary little between years (Pen *et al.*, 2010).

In this thesis I utilise data collected between 2000/1 and 2015/16, which consists of over 1200 litters from over 600 unique females, from each population. Because many of these females were themselves born in the laboratory during this period they have a known mother and siblings and we have captured many females in multiple years. Long-term datasets, tracking multiple populations, such as this, are

rare in any taxon, and are rarer still in reptile systems. Because this dataset extends over a 16-year period, across climatic extremes, and includes pedigree information, it provides an exceptional tool for investigating climatic determinants of intraspecific variation in phenotypically plastic traits. By using this dataset in combination with manipulative experiments and simulation modelling, I have been able to investigate the effect of climate on two key, and interlinked, phenotypically plastic traits that have significant consequences for population viability in this species, and which are likely to be affected by altered climatic conditions: the timing of parturition (i.e., birth phenology) and sex determination.

In this thesis I had two primary goals:

1) To investigate the climatic drivers of intraspecific patterns, causes and consequences of plasticity in two interlinked traits in *N. ocellatus* that have important consequences for population persistence, especially in the light of changing climates: parturition phenology and offspring sex ratios.

2) To investigate how climate affects geographical and temporal variation in patterns of selection for modes of sex determination in *N. ocellatus*, and the selective pressures that have led to the evolution of among-population divergence in sex determination systems.

Thesis presentation

This thesis includes five data chapters, a general discussion and an appendix. Chapters 2-4 have been published in peer-reviewed scientific journals, whilst chapters 5 and 6 are currently in preparation for submission. Thus, all chapters are presented as stand-alone documents and, by necessity, there is some repetition of information among them, especially in details of the study system and the long-term monitoring program. I am a lead author on all manuscripts, and performed all of the statistical analyses, but have recognised the importance of my collaborators' valuable

contributions by acknowledging them as co-authors. I share joint first authorship, with colleagues, of Chapters 2 and 4, for which I performed the statistical analyses and contributed equally to the preparation of the manuscripts. Because all chapters were formatted according to each journal's specifications, they differ from each other in aspects of their presentation. Finally, during my thesis I had the opportunity to contribute towards a paper, which does not relate directly to the content of this thesis. I have included this paper as an appendix. In response to reviewer feedback, several statistical models (Chapters 2–4) have been re-run with altered models. Rather than present these models in the text of each chapter, which would have resulted in differences between them and the published papers, I have included these models, and discussion of differences between their results and those in the main text, as an appendix to each of these chapters.

Chapter 2: Climate and sex ratio variation in a viviparous lizard

(Published as: Cunningham, While and Wapstra (2017). Climate and sex ratio variation in a viviparous lizard. *Biology Letters*, 13: 20170218.)

In chapter two, I investigate variation in offspring sex ratios and parturition dates in two climatically distinct populations of the spotted snow skink, *Niveoscincus ocellatus*, using a 16-year long-term dataset to establish whether these populations vary in their response to yearly temperature. I demonstrate that, in these populations, environmental temperatures have a similar effect on parturition dates, which occur earlier in warm years than in cold years. Conversely, the effect of yearly temperature on offspring sex is highly population-specific. In the lowland population females are more common in warm years and males are more common in cold years. In the highland population, however, offspring sex is not affected by temperature and offspring sex ratios vary little between years. These results confirm that these populations differ in the effect of temperature on offspring sex and suggest that these affects are independent of climatic effects on other physiological processes.

Chapter 3: Disentangling sex allocation in a viviparous reptile with temperature-dependent sex determination.

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Tests of sex allocation often consider only single causative factors, ignoring the potential for multiple factors to interact. In chapter 3, I investigate whether the resources available to mothers affect sex allocation decisions in the lowland population of *N. ocellatus* at various stages of the reproductive cycle in addition to, or in interaction with, the effects of temperature discussed in chapter 2. In this chapter, I use a multi-factorial approach to examine sex allocation in *N. ocellatus* by integrating data from the long-term field study with a manipulative laboratory experiment. Confirming my results from chapter 2, I demonstrate strong effects of temperature on sex allocation in the field, with females born in warm conditions and males in cold conditions; however, this was not replicated in the laboratory. In contrast, I found no effect of female resource availability on sex allocation, either independently, or in interaction with temperature. These results conformed to an overall lack of an effect of resource availability on any of the life history traits that I predicted would mediate the benefits of differential sex allocation in this system, suggesting that selection for sex allocation in response to resource availability may be relatively weak. Combined, my results suggest that temperature may be the predominant factor driving sex allocation in this system.

Chapter 4: Plastic rates of development and the effect of thermal extremes on offspring fitness in a cold-climate viviparous lizard.

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Populations at the climatic margins of a species' distribution can be exposed to conditions that cause developmental stress, resulting in developmental abnormalities. Even within the thermal range of normal development, phenotypes often vary with developmental temperature (i.e. thermal phenotypic plasticity). These effects can have significant consequences for organismal fitness and, thus, population persistence.

Understanding the extent and direction of thermal effects on phenotypes and their fitness consequences is crucial if we are to make meaningful predictions of how populations and species will respond as climates warm. In chapter 4, I use a combination of long-term data and a laboratory experiment to examine the consequences of thermal conditions at the margins of the highland population's normal temperature range. In this chapter I show that thermal conditions have substantial effects on phenotypic development in this population. Specifically, I demonstrate that offspring born earlier as a result of high environmental temperatures during the gestational period had increased growth over the first winter of life, but that there was no effect on offspring survival, nor was there an effect of developmental temperature on the incidence of developmental abnormalities.

Chapter 5: Degrees of change: between and within population variation in thermal reaction norms of phenology in a viviparous lizard.

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As climates warm, species, and the populations that make them up, will be faced with novel climatic environments to which they may not be adapted. Populations can be buffered against the negative effects, or maximise the beneficial effects, of altered conditions, in the short-term via phenotypic plasticity and/or in the longer term, through adaptive evolution. However, due to local adaptation and biogeographic and demographic history, populations are likely to differ in the shape of reactive norms, and the degree to which these vary between individuals at the phenotypic and the genetic level. If we are to accurately predict the consequences of climatic change for species and population persistence, we require estimates of population-specific patterns of variation. In chapter 5, I use the long-term dataset to investigate how patterns of variability in thermal reaction norms of parturition date vary within and between populations using an animal model framework. I demonstrate that, whilst the two populations share a common population-mean reaction norm (as shown in chapter 2), they differ substantially in the among-individual variability of reaction norms at the phenotypic level. Thus, these populations may be affected differently by changing climates.

Chapter 6: Sex in a warming climate: modelling patterns of sex determination across changing climatic landscapes.

(In preparation for later submission to Nature Ecology and Evolution as: Cunningham, Schwanz, While and Wapstra (in prep). Sex in a warming climate: modelling patterns of sex determination across changing climatic landscapes.)

Whilst a number of conceptual models have been proposed to explain the selective pressures that have led to the evolution and maintenance of sex determination systems and transitions between them, how these models translate to patterns across complex climatic landscapes remains, largely, unaddressed. In chapter 6, I present the results of an individual-based evolutionary simulation model, which draws together key findings from previous chapters, and is parameterised with data from our long-term program, to investigate how differing selective pressures have led to the evolution of distinct patterns of sex determination in *N. ocellatus*. This model builds on a previous conceptual model, which describes the evolution of these systems within two specific climatic contexts, so that these patterns can be estimated across the entire present day climatic landscape of Tasmania. By integrating this predictive model with downscaled climate predictions, I show how patterns of selection and sex ratios throughout the species' range will be altered as climates change.

Chapter 7: General discussion

In chapter 7, I discuss my findings in the context of research into phenotypically plastic traits, specifically focusing on thermal developmental plasticity, including thermal effects on phenology and sex determination. In this chapter I discuss how my research has informed the field, and suggest avenues of further research.

References

Anderson, S. (1984) Aerography of North American fishes, amphibians, and reptiles. *American Museum Novitates*, 2802, 1-6.

Anderson, S., and Markus, L.F. (1992) Aerography of Australian tetrapods. *Australian Journal of Zoology*, **40**: 627-651.

Araújo, M.B., Thuiller, W. & Pearson, R.G. (2006) Climate warming and the decline of amphibians and reptiles in Europe. *Journal of Biogeography*, **33**: 1712-1728.

Beebee, T.J.C. (1995) Amphibian breeding and climate. *Nature*, **374**: 219-220.

Beldade, P., Mateus, A.R.A. & Keller, R.A. (2011) Evolution and molecular mechanisms of adaptive developmental plasticity. *Molecular Ecology*, **20**: 1347-1363.

Böhm, M., Collen, B., Baillie, J.E.M, Bowles, P., Chanson, J., Cox, N., Heammerson, G., Hoffmann, M., Livingstone, S.R., Ram, *et al.* (2013) The conservation status of the world's reptiles. *Biological Conservation*, **157**: 372-385.

Böhm, M., Cook, D., Ma, H., Davidson, A.D., Garcia, A., Tapley, B., Pearce-Kelly, P. & Carr, J. (2016) Hot and bothered: using trait-based approaches to assess climate change vulnerability in reptiles. *Biological Conservation*, **204**: 32-41.

Bonebrake, T.C., Brown, C.J., Bell, J.D., Blanchard, J.L., Chauvenet, A., Champion, C., Chen I-C., Clark, T.D., Colwell, R.K., Danielsen, F. *et al.* (2018) Managing consequences of climate-driven species redistribution requires integration of ecology, conservation and social science. *Biological Reviews*, **93**: 284-305.

Both, C., van Asch, M., Bijlsma, R.G., van den Burg, A.B. & Visser, M.E. (2009) Climate change and unequal phenological changes across four trophic levels: constraints or adaptations? *Journal of Animal Ecology*, **78**: 73-83.

Boyle, M., Hone, J., Schwanz, L.E. & Georges, A. (2014) Under what conditions do climate-driven sex ratios enhance versus diminish population persistence? *Ecology and Evolution*, **4**: 4522-4533.

Bull, J.J. & Bulmer, M.G. (1989) Longevity enhances selection of environmental sex determination. *Heredity*, **63**: 315-320.

Cadby, C.D., Jones, S.M., & Wapstra, E. (2014). Geographical differences in maternal basking behaviour and offspring growth rate in a climatically widespread viviparous reptile. *Journal of Experimental Biology*, **217**: 1175-1179.

Caldwell, A.J., While, G.M., Wapstra, E. (2017) Plasticity of thermoregulatory behaviour in response to the thermal environment by widespread and alpine reptile species. *Animal Behaviour*, **132**: 217-227.

Chen, I.C., Hill, J.K., Ohlemuller, R., Roy D.B. & Thomas C.D. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science*, **333**: 1024-1026.

Chevin, L.M., Lande, R. (2010) Adaptation, plasticity and extinction in a changing environment: towards a predictive theory. *PLOS Biology*, **8**: e1000357.

Chevin, L.M., Collins, S., Lefevre, F. (2013) Phenotypic plasticity and evolutionary demographic responses to climate change: taking theory out to the field. *Functional Ecology*, **27**: 966-979.

Chevin, L.-M. & Hoffmann, A.A. (2017) Evolution of phenotypic plasticity in extreme environments. *Proceedings of the Royal Soeciety B – Biological Sciences*, **371**: 1723.

Cliff, H.B., Wapstra, E., & Burridge, C.P. (2015). Persistence and dispersal in a Southern Hemisphere glaciated landscape: the phylogeography of the spotted snow skink (*Niveoscincus ocellatus*) in Tasmania. *BMC evolutionary biology*, **15**: 121.

Cohen, J.M., Lajeunesse, M.J. & Rohr, J.R. (2018) A global synthesis of animal phenological responses to climate change. *Nature Climate Change*, **8**: 224-228.

Coulson, T., Milner-Gulland, E.J. & Clutton-Brock, T. (2000) The relative roles of density and climatic variation on population dynamics and fecundity rates in three contrasting ungulate species. *Proceedings of the Royal Society B – Biological Sciences*, **267**: 1771-1779.

Crick, H.Q.P, Dudley, C., Glue, D.E. & Thomson, D.L. (1997) UK birds are laying eggs earlier. *Nature*, **388**: 526.

Diffenbaugh, N.S. & Field, C.B. (2013) Changes in ecologically critical terrestrial climate conditions. *Science*, **341**: 486-492.

Doody, J.S., Guarino, E., Georges, A., Corey, B., Murray, G. & Ewert, M. (2006) Nest site choice compensates for climate effects on sex ratios in a lizard with environmental sex determination. *Evolutionary Ecology*, **20**: 307-330.

Durant, J.M., Hjermann, D.O., Ottersen, G. & Stenseth, N.C. (2007) Climate and the match or mismatch between predator requirements and resource availability. *Climate Research*, **33**: 271-283.

Escobedo-Galvan, A. H. & Gonzalez-Salazar, C. (2012) Survival and extinction of sex-determining mechanisms in Cretaceous tetrapods. *Cretaceous Research*, **36**:116–118.

Fitter, A.H. & Fitter, R.S.R. (2002) Rapid changes in flowering time in British plants. *Science*, **296**: 1689-1691.

García-Ramos, G. & Kirkpatrick, M. (1997). Genetic models of adaptation and gene flow in peripheral populations. *Evolution*, **51**: 21–28.

Ge, Q.S., Wang, H.J., Rutishauser, T. & Dai, J.H. (2016) Phenological responses to climate change in China: a meta-analysis. *Global Change Biology*, **21:** 265-274.

Ghalambor, C.K., McKay, J.K., Carroll, S.P. & Reznick, D.N. (2007) Adaptive versus non-adaptive plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, **21**: 395-407.

Gomez-Mestre, I. & Jovani, R. (2013) A heuristic model on the role of plasticity in adaptive evolution: plasticity increases adaptation, population viability and genetic variation. *Proceedings of the Royal Society B – Biological Sciences*, **280**: 20131869.

Hartmann, D.L., Klein Tank, A.M.G., Rusticucci, M., Alexander, L.V., Brönnimann, S., Charabi, Y., Dentener, F.J., Dlugokencky, E.J., Easterling, D.R., Kaplan, *et al.* (2013) 2013: Observations: Atmosphere and Surface. In: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* [Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V. and Midgley, P.M. (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Hays, G.C., Mazaris, A.D., Schofield, G. & Laloe, J.O. (2017) Population viability at extreme sex-ratio skews produced by temperature-dependent sex determination. *Proceedings of the Royal Society B – Biological Sciences*, **284**: 1848.

Hill, P.L., Burridge, C.P., Ezaz, T., Wapstra, E. (2018) Conservation of sex-linked markers among conspecific populations of a viviparous skink, *Niveoscincus ocellatus*, exhibiting genetic and temperature-dependent sex determination. *Genome Biology and Evolution*, 10: 1079-1087.

Hoffmann, A.A. & Sgró, C.M. (2011) Climate change and evolutionary adaptation. *Nature*, **470**: 479-485.

Husby, A., Nussey, D.H., Visser, M.E., Wilson, A.J., Sheldon, B.C. & Kruuk, L.E.B. (2010) Contrasting patterns of phenotypic plasticity in reproductive traits in two great tit (*Parus major*) populations. *Evolution*, **64**: 2221-2237.

Hüppop, O. & Hüppop, K. (2003) North Atlantic oscillation and timing of spring migration in birds. *Proceedings of the Royal Society B – Biological Sciences*, **270**: 233-240.

Intergovernmental Panel on Climate Change (IPCC) (2014) *Climate change 2014: Synthesis report, Contribution of working groups I, II and III to the Fifth assessment report of the Intergovernmental Panel on Climate Change*.

Janzen, F.J. (1994) Climate change and temperature-dependent sex determination in reptiles. *Proceedings of the National Academy of Science*s, **91**: 7487-7490.

Kawecki, T.J. & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, **7**: 1225–1241.

Kearney, M. & Porter, W. (2009) Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. *Ecology Letters*, **12**: 334-350.

Kemp, D.B., Eichenseer, K. & Kiessling, W. (2015) Maximum rates of climate change are systematically underestimated in the geological record. *Nature Communications*, **6**: 8890.

Lancaster, L.T., Morrison, G. & Fitt, R.N. (2017) Life history trade-offs, the intensity of competition, and coexistence in novel and evolving communities under climate change. *Philosophical Transactions of the Royal Society B – Biological Sciences*, **372**: 20160046.

Le Galliard, J.F., Fitze, P.S., Ferriere, R., & Clobert, J. (2005) Sex ratio bias, male aggression, and population collapse in lizards. *Proceedings of the National Academy of Sciences*, **102**: 18231-18236.

Lenoir, J., Gegout, J.C., Guisan, A., Vittoz, P., Wohgemuth, T., Zimmerman, N.E., Dullinger, S., Pauli, H., Willner, W. & Svenning, J.-C. (2010) Going against the flow: potential mechanisms for unexpected downslope range shifts in a warming climate. *Ecography*, **33**: 295-303.

Lenoir, J. & Svenning, J.-C. (2015) Climate-related range shifts – a global multidimensional synthesis and new research directions. *Ecography*, **38**: 15-28.

Luh, P.E.K., Jones, S.M., Wapstra, E. (2015) Energy expenditure of the spotted snow skink, *Niveoscincus ocellatus*, at two climatic extremes of its distribution range. *Journal of Thermal Biology*, **52**: 208-216.

Mainwaring, M.C., Barber, I., Deeming, D.C., Pike, D.A., Roznik, E.A. & Hartley, I.R. (2017) Climate change and nesting behaviour in vertebrates: a review of the ecological threats and potential for adaptive responses. *Biological Reviews*, **92**: 1991-2002.

Mitchell, N.J. & Janzen, F.J (2010) Temperature-dependent sex determination and contemporary climate change. *Sexual Development*, **4**: 129-140.

Menzel, A. & Fabian, P. (1999) Growing season extended in Europe. *Nature*, **397**: 659.

Menzel, A., Sparks, T.H., Estrella, N., Koch, E., Aasa, A., Ahas, R., Alm-Kubler, K., Bissolli, P., Braslavska, O., Briede, A. *et al.* (2006) European phenological response to climate change matches the warming pattern. *Global Change Biology*, **12**: 1969–1976.

Mitchell, N.J., Kearney, M.R., Nelson, N.J. & Porter, W.P. (2008). Predicting the fate of a living fossil: how will global warming affect sex determination and hatchling phenology in tuatara? *Proceedings of the Royal Society of London B: Biological Sciences* **275**: 2185–2193.

Neuwald, J.L. & Valenzuela, N. (2011) The lesser known challenge of climate change: thermal variance and sex- reversal in vertebrates with temperature-dependent sex determination. PLoS ONE, **6**:e18117.

Nicotra, A.B., Hermes, J.P., Jones, C.S. & Schlichting, C.D. (2007) Geographic variation and plasticity to water and nutrients in *Pelargonium australe*. **176**: 136-149.

Noble, D.W.A, Stenhouse, V. & Schwanz, L.E. (2018) Developmental temperatures and phenotypic plasticity in reptiles: a systematic review and meta-analysis. *Biological Reviews*, **93**: 72-97.

Nussey, D.H., Wilson, A.J. & Brommer, J.E. (2007) The evolutionary ecology of individual phenotypic plasticity in wild populations. *Journal of Evolutionary Biology*, **20**: 831-844.

Parmesan, C. (2007) Influences of species, latitudes and methodologies on estimates of phenological response to global warming. *Global Change Biology*, **13**: 1860–1872.

Parmesan, C. & Yohe, G. (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, **421**: 37–42.

Pecl, G.T., Arujo, M.B., Bell, J.D., Blanchard, J., Bonebrake, T.C., Chen, I.C., Clark, T.D., Colwell, R.K., Danielsen, F., Evengard, B. *et al.* (2017) Biodiversity redistribution under climate change: Impacts on ecosystems and human well-being. *Science*, **355**: 6332.

Post, E., Steinman, B.A. & Mann, M.E. (2018) Acceleration of phenological advance and warming with latitude over the past century. *Scientific Reports*, **8**: 3927.

Reed, T.E., Waples, R.S., Schindler, D.E., Hard, J.J. & Kinnison, M.T. (2010) Phenotypic plasticity and population viability: the importance of environmental predictability. *Proceedings of the Royal Society B – Biological Sciences*, **277**: 3391-3400.

Root, T.L., Price, J.T., Hall, K.R., Schneider, S.H., Rosenzweig, C. & Pounds, J.A. (2003) Fingerprints of global warming on wild animals and plants. *Nature*, **421**, 57–60.

Sabath, N., Itescu, Y, Feldman, A., Meiri, S., Mayrose, I. & Valenzuela, N. (2016) Sex determination, longevity and the birth and death of reptilian species. *Ecology and Evolution*, **6**: 5207-5220.

Saether, B.E., Lande, R., Engen, S., Weimerskirch, H., Lillegård, M., Altwegg, R., Becker, P.H., Bregnballe, T., Brommer, J.E., McCleery, R.H. *et al.* (2005) Generation time and temporal scaling of bird population dynamics. *Nature*, **436**: 99-102.

Scheiner, S.M. (1993) Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics*, **24**: 35-68.

Schwanz, L.E. & Janzen, F.J. (2008) Climate change and temperature-dependent sex determination: can individual plasticity in nesting phenology prevent extreme sex ratios? *Physiological and Biochemical Zoology*, **81**: 826-834.

Schwanz, L.E., Spencer, R.-J. Bowden, R.M. & Janzen, F.J. (2010) Climate and predation dominate juvenile and adult recruitment in a turtle with temperature-dependent sex determination. *Ecology*, **91**: 3016-3026.

Schwanz, L.E. (2013) Revealing the links between climate and demography for reptiles with environmental sex determination. *Animal Conservation*, **16**: 495-497.

Schwarzkopf, L. & Shine, R. (1991) Thermal biology of reproduction in viviparous skinks, *Eulamprus tympanum*: why do gravid females bask more? *Oecologia*, **88**: 562-569.

Schwarzkopf, L. & Andrews, R.M. (2012) Are moms manipulative or just selfish? Evaluating the "maternal manipulation hypothesis" and implications for life-history studies of reptiles. *Herpetologica*, **68**: 147-159

Shine, R (2012) Manipulative mothers and selective forces: the effects of reproduction on thermoregulation in reptiles. *Herpetologica*, **68**: 289-298.

Sinervo, B., Mendez-de-la-Cruz, F., Miles, D.B., Heulin, B., Bastiaans, E., Cruz, M.V. S., Lara-Resendiz, R., Martinez-Mendez, N., Calderon-Espinosa, M.L., Meza-Lazaro, R.N. *et al.* (2010) Erosion of lizard diversity by climate change and altered thermal niches. *Science*, **328**: 894-899.

Sparks, T.H., Roy, D.B. & Dennis, R.L.H. (2005) The influence of temperature on migration of Lepidoptera into Britain. *Global Change Biology*, **11**: 507-514.

Thackery, S.J., Henrys, P.A., Hemming, D., Bell, J.R., Botham, M.S., Burthe, S., Helaouet, P., Johns, D.G., Jones, I.D., Leech, D.I. *et al.* (2016) Phenological sensitivity to climate across taxa and trophic levels. *Nature*, **535**: 241-245.

The tree of sex consortium (2014) Tree of sex: a database of sexual systems. *Scientific Data*, 1:140015

Tuberville, T.D., Andrews, K.M., Sperry, J.H. & Grosse, A.M. (2015) Use of NatureServe climate change vulnerability index as an assessment tool for reptiles and amphibians: lessons learned. *Environmental Management*, **56**: 822-834.

Uller, T. & Olsson, M. (2008) Multiple paternity in reptiles: patterns and processes. *Molecular Ecology*: **17**: 2566-2580.

Uller, T., While, G.W., Cadby, C.D., Harts, A., O'Connor, K., Pen, I., & Wapstra, E. (2011). Altitudinal divergence in thermoregulatory behaviour may be driven by differences in selection on offspring survival in a viviparous lizard. *Evolution*, **65**: 2313-2324.

Ummenhofer, C.C. & Meehl, G.A. (2017) Extreme weather and climate events with ecological relevance: a review. *Philosophical Transactions of the Royal Society B – Biological Sciences*, **372**: 20160135.

Urban, M.C., Bocedi, G., Hendry, A.P., Mihoub, J.B., Pe'er, G., Singer, A., Bridle, J.R., Crozier, L.G., De Meester, L., Godsoe, W. *et al.* (2016) Improving the forecast for biodiversity under climate change. *Science*, **353**: 1113+

Valladares, F., Matesanz, S., Guilhaumon, F., Araujo, M.B., Balaguer, L., Benito-Garzon, M., Cornwell, W., Gianoli, E., van Kleunen, M., Naya, D.E. *et al.* (2014) The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecology Letters*, **17**: 1351-1364.

Visser, M.E. & Holleman, L.J.M. (2001) Warmer springs disrupt the synchrony of oak and winter moth phenology. *Proceedings of the Royal Society B – Biological Sciences*, **268**: 289-294.

Visser, M.E. (2008) Keeping up with a warming world; assessing the rate of adaptation to climate change. *Proceedings of the Royal Society B – Biological Sciences*, **275**: 649-659.

Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, J.M., Hoegh-Guldberg, O. & Bairlein, F. (2002) Ecological responses to recent climate change. *Nature*, **416**: 389-395

Wapstra, E. & Swain, R. (1996). Feeding ecology of the Tasmanian spotted skink, *Niveoscincus ocellatus* (Squamata: Scincidae). *Australian Journal of Zoology*, **44**: 205-213.

Wapstra, E. Swain, R. Jones, S.M. & O-Reilly, J. (1999) Geographic and annual variation in reproductive cycles in the Tasmanian spotted snow skink, *Niveoscincus ocellatus* (Squamata: Scinicidae). *Australian Journal of Zoology*, **47**: 359-550.

Wapstra, E. & Swain, R. (2001). Geographic and annual variation in life-history traits in a temperate zone Australian skink. *Journal of Herpetology*, **35**: 194-203.

Wapstra, E., Swain, R. & O'Reilly, J.M. (2001) Geographic variation in age and size at maturity in a small Australian viviparous skink, *Copeia*, **3**: 646-655.

Wapstra, E., Uller, T., Sinn, D.L., Olsson, M., Mazurek, K., Joss, J. & Shine, R. (2009). Climate effects on offspring sex ratio in a viviparous lizard. *Journal of Animal Ecology*, **78**: 84-90.

Wedekind, C. (2002) Manipulating sex ratios for conservation: short term risks and long-term benefits. *Animal Conservation*, **5**: 13-20.

While, G.M. & Uller, T. (2014) Quo vadis amphibia? Global warming and breeding phenology in frogs, toads and salamanders. *Ecography*, **37**: 921-929.

Zhang, L., Takahashi, D., Hartvig, M. & Andersen, K.H. (2017) Food-web dynamics under climate change. *Proceedings of the Royal Society B – Biological Sciences*, **284**: 1867.

Chapter 2:

Climate and Sex Ratio Variation in a Viviparous Lizard

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Abstract

The extent to which key biological processes, such as sex determination, respond to environmental fluctuations is fundamental for assessing species' susceptibility to ongoing climate change. Few studies address how climate affects offspring sex in the wild. We monitored two climatically distinct populations of the viviparous skink *Niveoscincus ocellatus* for 16 years, recording environmental temperatures, offspring sex and date of birth. We found strong population-specific effects of temperature on offspring sex, with female offspring more common in warm years at the lowland site but no effect at the highland site. In contrast, date of birth advanced similarly in response to temperature at both sites. These results suggest strong population-specific effects of temperature on offspring sex that are independent of climatic effects on other physiological processes. These results have significant implications for our understanding of the ecological and evolutionary consequences of variation in sex ratios under climate change.

1. Introduction

Climate affects the phenology, physiology and behaviour of many organisms and acts as an important driver of evolutionary and ecological change. Understanding these effects has never been more important, given the need to predict how species and ecosystems will to respond changing climates. How the environment influences offspring sex is particularly important in this context, as extreme sex ratio skews may lead to rapid demographic collapse [1, 2, 3, 4]. We understand how sex ratios respond to climate in the wild, however, for only a handful of species [e.g., 1, 5, 6].

Here we use an extensive 16-year dataset from two populations of the viviparous lizard *Niveoscincus ocellatus* to investigate how climate affects offspring sex and birth date. Previously, we have demonstrated with theoretical models intraspecific climatic divergence in sex determination in this system [7]. Crucial to further understanding the mechanisms underlying this divergence and its consequences under directional climate change, are long-term datasets that explore population-level reaction norms. We address this by examining how variation in temperature influences offspring sex ratios and phenology in population-specific ways. We discuss the consequences of population-specific responses for population persistence in the light of changing climates.

2. Materials and methods

Niveoscincus ocellatus is a small short-lived viviparous lizard, endemic to Tasmania, with a wide climatic and altitudinal range [7, 8, 9]. We monitored two populations, located at the extreme limits of the species thermal and altitudinal distribution, annually for the past 16 years (2000/2001 to 2015/2016). The lowland population is located on the East Coast (42°55'S, 147°87'E, elevation: 30 m), whilst the highland population is located on the Central Plateau (41°86'S, 146°53'E, elevation: 1150 m). Both sites are self-contained with little immigration or emigration [9, 10] and ovulation is highly synchronised among females within sites [11]. Each year the majority of pregnant females were captured at the end of gestation (December and January for the lowland and highland sites respectively) to minimise time spent in the laboratory and well after the critical sex-determining period, which occurs during the

first half of gestation [11,12]. Females were housed following standard laboratory protocols [8, 9, 11]. At birth we recorded morphometric traits, birth date and sex of offspring prior to release at their site of origin. The dataset consists of more than 1100 litters from each site (Table 1).

Table 1. Number of litters, individual offspring and cohort sex ratio (proportion of males) in lowland and highland *N. ocellatus* (2000 – 2015).

	Highland population			Lowland population		
Season	Number of litters	Number of offspring	Cohort Sex ratio	Number of litters	Number of offspring	Cohort Sex ratio
2000/2001	88	373	0.536	91	196	0.449
2001/2002	114	438	0.547	99	222	0.590
2002/2003	83	294	0.561	90	205	0.561
2003/2004	102	351	0.493	104	211	0.664
2004/2005	99	318	0.475	93	220	0.586
2005/2006	96	317	0.527	110	283	0.445
2006/2007	85	261	0.537	91	221	0.486
2007/2008	73	242	0.579	71	171	0.500
2008/2009	59	204	0.559	92	233	0.532
2009/2010	43	160	0.525	98	242	0.529
2010/2011	45	163	0.509	104	253	0.530
2011/2012	48	176	0.520	92	227	0.493
2012/2013	52	187	0.503	87	190	0.503
2013/2014	71	276	0.509	79	200	0.600
2014/2015	71	269	0.506	72	178	0.489
2015/2016	62	237	0.477	50	127	0.548
Total	1191	4266	0.523	1423	3379	0.530

To examine responses of offspring sex and birth date to climate, we used temperature during the first half of gestation, which corresponds to the critical sex-determining period (T_{crit}), [11, 12]. Due to minor temporal variation in timing of gestation between sites [8, 9] this corresponded to the mean maximum daytime temperature (from Australian Bureau of Meteorology stations) from October 1-November 14 and October 15 - November 31 at the lowland and highland sites respectively. The effect of T_{crit}, site and their interaction on offspring sex ratio of

litters was tested by fitting a generalised linear mixed model (GLMM) with binomial error distribution (logit link) using a type II Wald X^2 test, whilst their effect on birth date was tested by fitting a linear mixed model (LMM). Both of these models were fit with maternal identity as a random effect to account for repeated sampling of mothers across years and with T_{crit} mean centred (in reduced, site-specific, models T_{crit} was mean centred by site; see below). Where no significant interaction effects were found we present test statistics and P-values of main effects from reduced models excluding interaction terms. Analyses were conducted using R [13] using the "lme4" package [14].

3. Results

We found a significant interaction effect of site and T_{crit} on offspring sex. At the lowland site each increase of 1°C of T_{crit} resulted in a 0.869 change in the odds of offspring being born male (figure 1a), i.e., a skew towards males at lower temperatures and towards females at higher temperatures. At the highland site there was no significant effect of temperature on offspring sex (table 2), and cohort sex ratios deviated little between years (figure 1a). There was also a significant interaction effect of site and T_{crit} on birth date (table 2; figure 1b). However, site-specific models showed a significant effect of T_{crit} at both sites (table 2), but with a more extreme effect at the highland site (advancing 4.92 ± 0.18 SE days for each 1°C increase in T_{crit}) compared with the lowland site (advancing 3.08 ± 0.38 SE for each 1°C increase in T_{crit}) (figure 1b).

Table 2: GLMM and LMM models assessing the effect of site and T_{crit} on offspring sex (GLMM) and birth date (LMM) in lowland and highland *N. ocellatus* (2000 – 2015). The interaction model includes both sites whilst site-specific models are run on data from each site, separately. Sex ratios in the GLMM model were performed at the level of sex ratios of litters.

	Site-specific models		
	Lowland T_{crit}	Highland T_{crit}	Site: T_{crit} interaction
Offspring sex	$\chi^2 = 6.392$ $P = 0.011$	$\chi^2 = 0.002$ $P = 0.967$	$\chi^2 = 5.619$ $P = 0.018$
Birth date	$F_{1,1237.2} = 67.22$ $P < 0.0001$	$F_{1,1070.9} = 788.34$ $P < 0.0001$	$F_{1,2306.1} = 17.39$ $P < 0.0001$

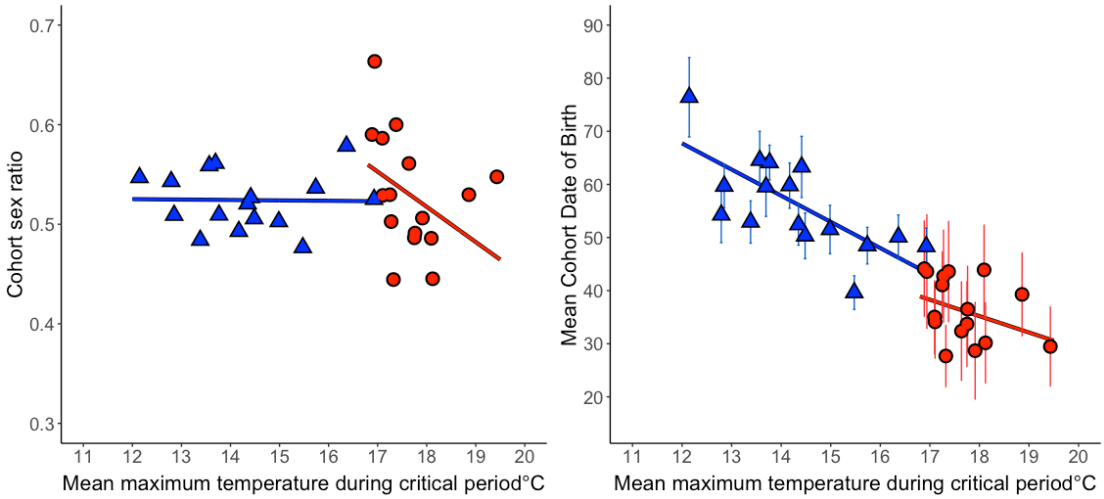

Figure 1. Relationships between mean maximum daytime temperature during gestation (T_{crit}) and (a) offspring sex (proportion of males) and (b) birth date, in lowland (circles) and highland (triangles) *N. ocellatus* (200- 2015).

4. Discussion

We found strong evidence for site-specific effects of temperature on offspring sex in two climatically distinct populations of *Niveoscincus ocellatus*. Lowland population sex ratios were skewed towards females in warmer years, and towards males in cooler years whereas, in the highlands, offspring sex was unaffected by temperature. These results are significant in two ways. First, they add to the limited number of studies demonstrating an effect of environmental temperature on offspring sex ratios in the wild, especially in short-lived, live-bearing reptiles. Second, they show that these effects are strongly population specific.

We have previously suggested that the divergence in the response of sex to temperature evolved as an adaptive response to local climate [7]. At the lowland site, a sex ratio response to temperature is adaptive because of the selective advantage that females, but not males, derive from being born early. In contrast, such a response is selected against at the highland site, where females gain no advantage from early birth and where high interannual variability would lead to maladaptive fluctuating cohort sex ratios. However, this evolutionary explanation does not address the mechanisms underlying the intraspecific divergence.

Our results suggest the divergence is not the result of differences in how individuals within the two populations buffer the effect of temperature on embryonic development through altered thermoregulatory behaviour. In *N. ocellatus*, as in other ectotherms, gestation length is temperature dependent [15] and the consistent link between environmental temperature and birth date suggests that the signal of environmental temperature on developing embryos is similar in both populations (potentially actually slightly stronger in the highland than the lowland population). Despite this we find strong site-specific effects of temperature on sex ratios. One explanation for these results is that the two populations share a similar sex ratio response to temperature, but that they appear different because sex ratio skews only occur at temperatures above those experienced by the highland population. However, our results suggest that this is not the case. First, where temperatures overlap between the populations (e.g., in the coldest years in the lowlands and warmest years in the highlands) sex ratios are male-skewed in the lowland population but not skewed in the highland population. Second, previous common garden experiments showed no effect of thermal conditions experienced at the lowland site on offspring sex ratios in highland females [7]. This suggests that the patterns observed are the result of fundamental differences in the temperature sensitivity of the mechanisms underlying sex determination. To determine the precise mechanisms underlying these effects, further research is required, focusing on the genetic and epigenetic differences between populations with divergent sex ratio responses to temperature.

What might the consequences of the relationship between offspring sex and temperature be for population persistence? Under directional climate change sex ratios are likely to remain stable in the highland but become increasingly female-skewed in the lowlands, and these effects will not be buffered by changes in thermoregulatory behaviour (analogous to buffering through varying nest depth in oviparous species [e.g., 4, 5]). Such shifts in sex ratios can have significant consequences for population persistence [5]. We suggest, however, that *N. ocellatus* is at comparatively low risk for a number of reasons (see [2]). First, high temperatures result in female-skewed sex ratios (an "MF" pattern). Therefore, as temperatures rise, population growth may actually be enhanced, as population growth is generally determined by the number of females, particularly in species where males may mate with multiple females, such as *N. ocellatus*, [16]. This remains true, however, only so

long as temperatures do not continue to rise to the point where cohort sex ratios are skewed to the extent that the number of males becomes limiting [17]. Second, the range of temperatures that produce mixed sex ratios (the transitional range of temperatures), is very broad. Therefore, given interannual fluctuations in temperature, there is a low probability of sequential single-sex or highly skewed cohorts that could lead to rapid demographic collapse. Third, the sex response in *N. ocellatus* varies between populations and there has been gene flow between populations in past instances of climate change [18]. Thus, populations may be able to adapt to changed climates relatively rapidly. Finally, a longer period before the onset of winter allows more time for growth and leads to increased survival and earlier maturity [19, 20]. Thus, whilst a sex ratio response to temperature is often considered to be a risk factor for population persistence as climates warm, populations of *N. ocellatus* in which sex ratios are affected by temperature are, nevertheless, at a comparatively low risk from changing climates.

This study demonstrates the importance of long-term studies, ideally across multiple, climatically distinct, populations to determine whether the responses of populations to temperature are consistent across species' ranges. Such information is crucial if we are to accurately predict the potential impact of climate change on species persistence.

Ethics: All guidelines and procedures for the use of animals were approved by the University of Tasmania Animal Ethics Committee (no. A0012087).

Data accessibility: The dataset is available at the Dryad digital repository: http://dx.doi.org/10.5061/dryad.hh67j [21]

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References

1. Janzen FJ. 1994 Climate change and temperature-dependent sex determination in reptiles. *Proc. Natl. Acad. Sci., USA*, **91**, 7487-7490. (doi: 10.1073/pnas.91.16.7487)

2. Mitchell NJ, Janzen FJ. 2010 Temperature-dependent sex determination and contemporary climate change. *Sexual Development* **4**, 129-140 (doi: 10.1159/000282494

3. Mitchell NJ, Kearney MR, Nelson NJ, Porter WP. 2008 Predicting the fate of a living fossil: how will global warming affect sex determination and phenology in tuatara? *Proc. R. Soc. B* **275**, 2185-2193. (doi: 10.1098/rspb.2008.0438)

4. Le Galliard JF, Fitze PS, Ferriere R, Colbert J. 2005 Sex ratio bias, male aggression, and population collapse in lizards. *PNAS* **102**, 18231-18236. (doi: 10.1073/pnas.0505172102)

5. Doody JS, Guarino F, Georges A, Corey B, Murray G, Ewart MW. 2006 Nest site choice compensates for climatic effects on sex ratios in a lizard with environmental sex determination. *Evol. Ecol.* **20**, 307-330. (doi: 10.1007/s10682-006-0003-2)

6. Schwanz LE, Spencer RJ, Bowden RM, Janzen FJ. 2010 Climate and predation dominate juvenile and adult recruitment in a turtle with temperature-dependent sex determination. *Ecology* **91**, 3016-3026. (doi: 10.1890/09-1149.1)

7. Pen I, Uller T, Feldmeyer B, Harts A, While GM, Wapstra E. 2010 Climate-driven population divergence in sex-determining systems. *Nature* **468**, 436-U262. (doi: 10.1038/nature09512)

8. Cadby CD, Jones SM, Wapstra E. 2014 Geographical differences in maternal basking behaviour and offspring growth in a viviparous reptile. *J. Exp. Biol.* **217**, 1175-1179. (doi: 10.1242/jeb.089953)

9. Uller T, While GM, Cadby CD, Harts A, O'Connor K, Pen I, Wapstra E. 2011 Altitudinal divergence in maternal thermoregulatory behaviour may be driven by differences in selection on offspring survival in a viviparous lizard. *Evolution* **65**, 2313-2324. (doi: 10.1111/j.1558-5646.2011.01303.x)

10. Cadby CD, While GM, Hobday AJ, Uller T, Wapstra E. 2010 Multi-scale approach to understanding climate effects on offspring size at birth and date of birth in a reptile. *Integr. Zool.* **5**, 164-175. (doi: 10.1111/j.1749-4877.2010.00201.x)

11. Wapstra E, Uller T, Sinn DL, Olsson M, Mazurek K, Joss J, Shine R. 2009 Climate effects on offspring sex ratio in a viviparous lizard. *J. Anim. Ecol.* **78**, 84-90. (doi: 10.1111/j.1365-2656.2008.01470.x)

12. Neaves L, Wapstra E, Birch D, Girling JE, Joss JMP. 2006 Embryonic gonadal and sexual organ development in a small viviparous skink, *Niveoscincus ocellatus*. *J. Exp. Zool. A* **305A**, 74-82. (doi: 10.1002/jez.a.249)

13. R Core Team. 2013 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. (http://www.R-project.org/)

14. Bates D, Maechler M. 2010 *lme4: linear mixed-effects models using S4 classes*. R package version 0.999375-33. See http://CRAN.R-project.org/package=lme4.

15. Wapstra, E 2000 Maternal basking opportunity affects juvenile phenotype in a viviparous lizard. *Functional Ecology* **14**, 345-352. (doi: 10.1046/j.1365-2435.2000.00428.x)

16. Wedekind C. 2002 Manipulating sex ratios for conservation: short term risks and long-term benefits. *Animal Conservation* **5**, 13-20. (doi: 10.1017/S1367943002001026)

17. Rankin, DJ, Kokko H. 2007 Do males matter? The role of males in population dynamics. *Oikos* **116**, 335-348. (doi: 10.1111/j.2006.0030-1299.15451.x.)

18. Cliff H, Wapstra E, Burridge CP. 2015 Persistence and dispersal in a Southern Hemisphere glaciated landscape: the phylogeography of the spotted snow skink (*Niveoscincus ocellatus*) in Tasmania. *BMC Evol. Biol.* **15**, 121-121. (doi: 10.1186/s12862-015-0397-y)

19. Wapstra E, Swain R, O'Reilly JM. 2001 Geographic variation in age and size at maturity in a small Australian viviparous skink. *Copeia* **3**, 646-655. (doi: 10.1643/0045-8511(2001)001[0646:GVIAAS]2.0.CO;2)

20. Wapstra E, Uller T, While GM, Olsson M, Shine R. 2010 Giving offspring a head start in life: field and experimental evidence for selection on maternal basking behaviour in lizards. *J. Evol. Biol.* **23** 651-657. (doi: 10.1111/j.1420-9101.2009.01924.x)

21. Cunningham GD, While, GM & Wapstra E. 2017 Data from: climate and sex ratio variation in a viviparous lizard. Dryad Digital Repository. (http://dx.doi.org/10.5061/dryad.hh67j)

Chapter 2, Appendix:

Models testing the effect of temperature during the critical period on date of birth (LMM) and offspring sex (GLMM), corresponding to the analysis presented in Table 2, but fit by maximum likelihood and with year included as a factorial random effect. Note, that significance of the effect of temperature on date of birth and offspring sex for the lowland site is non-significant in these tests, differing from the statistical significance of these effects presented in the main text and the published paper (Cunningham *et al.*, 2017). However, because all individuals within each site had the same measure for T_{crit} within years, temperature and year were inescapably confounded in site-specific models. Estimates of the effect (βs) of temperature on both date of birth and offspring sex at the lowland site did not differ substantially from estimates in the models fit without year as a random effect presented in the main text. Thus, although the statistical significance differed when year was included as a random effect, this is likely a consequence of confounding between year and temperature. Consequently the analysis presented in this chapter should be accepted as a better test of the significance of temperature on date of birth and offspring sex.

Table 2: GLMM and LMM models assessing the effect of site and T_{crit} on offspring sex (GLMM) and birth date (LMM) in lowland and highland *N. ocellatus* (2000 – 2015) with year included as a random effect, and with the significance of main effects tested for by maximum likelihood. Note, the β estimate of the effect of temperature on offspring sex is presented on the log(odds) scale. The interaction model includes both sites whilst site-specific models are run on data from each site, separately. Sex ratios in the GLMM model were performed at the level of sex ratios of litters.

	Site-specific models		
	Lowland T_{crit}	Highland T_{crit}	Site: T_{crit} interaction
Offspring sex	β = -0.12 ± 0.08 SE $\chi^2_{1,N=1423}$ = 2.215 P = 0.137 σ_{ID} = 0.02 ± 0.14 SD σ_{Year} = 0.03 ± 0.16 SD	β = 0.00 ± 0.02 SE $\chi^2_{1,N=1191}$ = 0.002 P = 0.967 σ_{ID} = 0.00 ± 0.00 SD σ_{Year} = 0.00 ± 0.00 SD	β = -0.15 ± 0.06 SE $X^2_{1,N=2614}$ = 5.484 P = 0.019 σ_{ID} = 0.00 ± 0.00 SD σ_{Year} = 0.00 ± 0.04 SD
Birth date	β = -2.76 ± 2.00 $F_{1, 16.1}$ = 1.95 P = 0.182 σ_{ID} = 39.16 ± 6.26 SD σ_{Year} = 27.80 ± 5.27 SD σ_{Resid} = 31.73 ± 5.63 SD	β = -4.68 ± 1.17 SE $F_{1, 16.0}$ = 16.11 P = 0.001 σ_{ID} = 10.35 ± 3.22 SD σ_{Year} = 35.46 ± 5.96 SD σ_{Resid} = 13.44 ± 3.66 SD	β = 1.12 ± 0.45 SE $F_{1, 2365.3}$ = 6.398 P = 0.012 σ_{ID} = 28.22 ± 5.31 SD σ_{Year} = 25.58 ± 5.06 SD σ_{Resid} = 32.40 ± 5.69 SD

Chapter 3:

Disentangling Sex Allocation in a Viviparous Reptile with Temperature-Dependent Sex Determination: a Multifactorial Approach

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Abstract

Females are predicted to alter sex allocation when ecological, physiological and behavioural variables have different consequences on the fitness of male and female offspring. Traditionally, tests of sex allocation have examined single causative factors, often ignoring possible interactions between multiple factors. Here we used a multi-factorial approach to examine sex allocation in the viviparous skink, *Niveoscincus ocellatus*. We integrated a 16-year observational field study with a manipulative laboratory experiment to explore whether the effects of the maternal thermal environment interact with the resources available to females for reproduction to affect sex allocation decisions. We found strong effects of temperature on sex allocation in the field, with females born in warm conditions and males in cold conditions, however, this was not replicated in the laboratory. In contrast, we found no effect of female resource availability on sex allocation, either independently, or in interaction with temperature. These results corresponded with an overall lack of an effect of resource availability on any of the life history traits that we predicted would mediate the benefits of differential sex allocation in this system, suggesting that selection for sex allocation in response to resource availability may be relatively weak. Combined, these results suggest that temperature may be the predominant factor driving sex allocation in this system.

Introduction

How a female divides her reproductive energy among male and female offspring is the key question underpinning sex allocation theory (Charnov, 1982; West, 2009; Wapstra & Warner, 2010). An unequal allocation of resources to male versus female offspring is expected either when extrinsic or intrinsic factors provide sex-specific effects on offspring fitness, or when the costs of producing male or female offspring differ (Fisher, 1930; Charnov, 1982). Significant sex allocation biases are not uncommon in nature, having been found in a wide range of taxa, including haplodiploid insects (Charnov, 1979), fish (Conover, 1984), lizards (Warner & Shine, 2008), birds (Komdeur, 1996) and mammals (Cameron & Dalerum, 2009). A major challenge for evolutionary biologists is to understand the factors that drive or constrain differential allocation of maternal resources between the sexes.

To address this challenge, empirical and theoretical biologists have attempted to explain how various factors, including the environment, demography, physiology and behaviour influence sex allocation patterns (Trivers & Willard, 1973; West, 2009). However, while these models have proved successful in predicting sex allocation in some systems (especially invertebrates; Seger & Stubblefield, 2002), the field is plagued by a lack of consistency in observed patterns of sex allocation among studies and limited agreement between theoretical predictions and empirical patterns (Frank, 1990; Wapstra & Warner, 2010). These inconsistencies may arise for several reasons. For example, the majority of sex allocation hypotheses focus on a single factor, ignoring the possibility that the multi-factorial complexities of life-histories in natural systems may obscure results if multiple factors interact (Cockburn *et al.,* 2002; Seger & Stubblefield, 2002). Second, the integration of observational and experimental studies is rare. This is problematic, as focussing solely on laboratory results may reveal sex allocation responses that are artefacts of an experimental environment (Frank, 1990; Komdeur & Pen, 2002) whilst observational studies provide only correlational evidence, and may misattribute the true causes of observed patterns.

In this study, we applied a multifactorial approach to examine sex allocation in the viviparous skink, *Niveoscincus ocellatus* (Gray, 1845). We have previously

demonstrated that offspring sex is affected by temperature in this species (Wapstra *et al.*, 2004; Wapstra *et al.*, 2009; Pen *et al*., 2010; Chapter 2: Cunningham *et al.*, 2017). In *N. ocellatus,* as in other ectotherms, warmer conditions result in earlier dates of birth because of accelerated embryonic development. This, in turn, results in a longer growth period in the first year of life, which enhances female, but not male, fitness (Wapstra, 2000; Pen *et al*., 2010; Wapstra *et al.*, 2010). Coupling offspring sex to temperature, then, provides a mechanism by which mothers maximize their fitness by skewing the sex ratio of litters towards females when high temperatures lead to early births, and towards males when low temperatures lead to later births (Pen *et al.*, 2010). Unlike other reptilian taxa in which offspring sex is affected by temperature (e.g., turtles and crocodiles), where the reaction norm of temperature effects on offspring sex is steep (i.e, 100% of each sex produced either side of a pivotal temperature), the reaction norm in *N. ocellatus* is shallow (Chapter 2: Cunningham *et al*., 2017). Furthermore, temperature alone does not account for the full variability in sex allocation patterns observed, suggesting that other factors may also affect these patterns. In other reptile species (Radder *et al.*, 2009), including the closely-related snow skink, *Niveoscincus microlepidotus* (Olsson & Shine, 2001), offspring size at birth has sex-specific fitness effects, with females benefitting more than males from being born large. Females that produce larger offspring may, therefore, also skew sex allocation towards females (a reverse of predictions made under classic Trivers – Willard (1973)). Thus, any factor that affects the ability of females to manipulate offspring size may also influence sex allocation decisions in addition to, or in interaction with, temperature.

In *N. ocellatus*, energetic allocation to offspring occurs in two key periods; prior to ovulation, during an extended period of vitellogenesis from soon after the previous birth using capital and income energy sources (Wapstra *et al.*, 1999) and throughout gestation via a complex chorioallantoic placenta (i.e., matrotrophy; Thompson *et al.*, 2001). *Niveoscincus* is one of only five lineages of squamate reptiles in which substantial placentotrophy occurs. Indeed, in *N. ocellatus*, neonates are more than 1.5 times larger in dry matter than freshly ovulated eggs (Thompson *et al*., 2001). Thus, offspring size at birth may be affected by resources available to females both prior to ovulation and during gestation. Resources available during either of

these stages may, therefore, influence sex allocation decisions. Importantly, such effects may interact with the known effects of temperature. For instance, female basking decisions may be altered by body condition (e.g., females in better condition may be able to devote more time to basking). Conversely, nutritional and energetic requirements of offspring may be influenced by temperature (since, in ectotherms, metabolism is temperature-dependent) or the process of nutrient assimilation across the placenta may itself be temperature-dependent (see Wapstra, 2000).

To explore the potential effect of resource availability on sex allocation decisions in *N. ocellatus*, we combined a manipulative laboratory experiment with data from a 16-year observational field study. We predicted that females producing larger offspring would overproduce daughters (and vice versa) and that, in line with previous results, females would produce female-skewed litters in warm years (field study) or when given access to increased basking opportunities (laboratory study) and male-skewed litters in cold years, or when given reduced basking opportunities. We also predicted that females in better condition at the beginning of vitellogenesis (field study) or at ovulation (laboratory study) would increase their reproductive effort. If this resulted in larger offspring (rather than increased number of offspring), then we predicted that these offspring would also be more likely to be female. In *N. ocellatus*, litter size is determined during vitellogenesis and is fixed at ovulation. We, therefore, predicted that additional resources provided to females after this time (i.e, during gestation; laboratory study) would lead to an increase in offspring size, and female-skewed litters. Because of the potential for temperature and resource availability to alter basking or investment decisions and, therefore, to have interacting effects on offspring size and dates of birth (and thus offspring sex), we also considered interactions between resource availability and temperature or basking in our analyses. Specifically, we predicted that when these factors acted in the same direction (i.e., low temperature and low resource availability would lead to male offspring or high temperature and high resource availability would lead to female offspring) the effects of the factors would reinforce each other. Conversely, when factors acted in different directions (i.e., low temperatures and high resource availability or high temperatures and low resource availability), effects on offspring sex would counteract each other

and the overall strength and direction of the response would depend on the competing magnitude of these effects.

Materials and Methods

Study Species

Niveoscincus ocellatus is a small viviparous skink (3–10 g, 60–75 mm snout-vent length (SVL)) endemic to Tasmania, Australia. For the past 16 years (since the austral summer of 2000/2001) a population located on the East Coast (42°55'S, 147°87'E) has been monitored, following a standard field and laboratory protocol (see Wapstra & O'Reilly, 2001; Wapstra *et al*., 2009; Chapter 2: Cunningham *et al.*, 2017). The study site is flanked on all sides by either roads or unsuitable habitat and, consequently, there is little immigration or emigration. At this site, animals are active from August through May and females reproduce annually (Wapstra *et al*., 1999). Mating occurs predominately in the Austral autumn (March to May), with a second mating period in spring, (late August to September) and females store sperm throughout winter (Jones *et al.*, 1997; While & Wapstra, 2009). Vitellogenesis commences soon after parturition (Wapstra *et al.*, 1999) and ovulation is highly synchronized within the population, occurring in early October each year (Wapstra *et al.*, 1999, 2009; Pen *et al.*, 2010; Uller *et al.*, 2011). Gestation length is variable among years, with births occurring earlier in warmer years and later in cooler years (Wapstra *et al.*, 2009; Cadby *et al.*, 2010; Uller *et al.*, 2011, Chapter 2: Cunningham *et al*., 2017). Females produce clutches of one to six offspring annually (though litters larger than four are rare), and there is no postnatal care (Wapstra *et al.*, 1999; Wapstra & Swain, 2001). Individuals reach maturity at two to three years of age and can live for more than seven years (Wapstra *et al.*, 2001; Pen *et al*., 2010).

Field study: animal capture and data collection

Each year pregnant females (up to 100 females per year, representing 90–95% of the adult female population) were caught towards the end of gestation in late December (well after the period of sex determination; Neaves *et al*., 2006; Wapstra *et al*., 2009).

Upon capture, females were identified using unique toe clips and their capture location was recorded before they were brought to a temperature-controlled laboratory (ambient temperature 16 °C) at the University of Tasmania. Females were then weighed (±1 mg) and transferred to individual plastic terraria (30 x 20 x 20 cm), each containing paper pellets as a substrate, a wooden cover for shelter and a rock for basking. A basking light was fitted over each terrarium to provide a thermal gradient for basking from 16 to 40 °C and water was available *ad libitum.* Three times per week all lizards were fed live insects (*Tenebrio* larvae) and crushed fruit (Heinz baby food) with added protein powder (Nature's Way) and terraria were checked twice daily for neonates.

At birth, offspring and mothers were measured (SVL ± 0.01 mm) and weighed (mass ± 0.1 mg), offspring and previously uncaught mothers toe-clipped and offspring sexed by hemipene eversion (Wapstra *et al.*, 2004; Wapstra *et al.*, 2009). Adult females were released within 5 m of their original site of capture, whilst offspring were randomly released at one of 12 locations within the study site. Climate data were obtained from a weather station located approximately 5 km from the study site ($42^{o}34$'S, $147^{o}52$'E) through the Australian Bureau of Meteorology website (Australian Government Bureau of Meteorology, 2015).

Field study: statistical analysis

Maternal body condition at the onset of vitellogenesis (C_{vit}) was used as a measure of the energy available for reproduction into the next litter prior to vitellogenesis. Each female's C_{vit} and postpartum condition (C_{pp}) was calculated as her residual mass from a regression model of mass on SVL measured immediately after parturition for all females across all years of the study (A female's C_{pp}, therefore equates to her C_{vit} in the following year). The first year of data (2000/2001), and females from subsequent years that did not give birth in the previous year were excluded when testing for the effect of C_{vit}, since no measure was available. In total, across the 16-year study, we collected data from 1395 litters across 673 unique females, and were able to calculate C_{vit} for the mothers of 576 litters (Supplementary Information; Table S1). Relative litter mass (RLM), calculated as the ratio of the total litter mass to the postpartum body mass of the mother, was used to estimate reproductive effort (Shine, 1980).

Cohort sex ratios presented in Figure 1 were calculated as the number of male offspring in a year divided by the total number of offspring in that year, such that values above 0.5 indicate male-skewed cohorts and values below 0.5 indicate female-skewed cohorts.

We assessed the effect of C_{vit}, mean maximum temperature throughout the gestation period (T_{gest}; October 1st to December 31st), and their interaction on mean litter offspring mass, RLM, date of birth and C_{pp} using general linear mixed models (LMMs), fit by REML. Maternal identity was included as a random effect, with random intercept and random slope in response to T_{gest} to account for repeated measures of mothers across years. To test whether C_{vit}, mean maximum temperature during the critical period of sex determination (T_{crit}; October 1st to November 15th (Neaves *et al.*, 2006; Wapstra *et al.*, 2009)) or their interaction had an effect on offspring sex, we fit a Generalised Linear Mixed Model (GLMM) with binomial error distribution (logit link) and maternal identity as a random effect with random intercept and random slope in response to T_{crit}, using a type II Wald X^2 test. In all analyses, independent variables other than C_{vit} were mean-centred. C_{vit} was not centred since the mean value was very close to zero, only deviating from this because postpartum maternal condition for the last year of the study (2015/2016) was used to establish the relationship and because some females used to establish the relationship did not give birth in the following year. We compared the mass of male and female offspring by fitting a LMM with offspring sex as a fixed factor and with litter identity (mother within year) nested within maternal identity as random effects. The overall sex ratio was calculated for all offspring born over the duration of the study and significance of deviation from equality was calculated using an exact binomial test.

Laboratory study: experimental procedures

One hundred and twenty-eight gravid *N. ocellatus* were collected from areas surrounding our field site in early October 2011. Females were collected, measured (SVL ± 1 mm) and weighed (± 1 mg) within the first weeks after ovulation (Wapstra *et al.*, 1999; Wapstra *et al*., 2009), but well prior to embryo sexual differentiation and sex determination (Neaves *et al.*, 2006; Wapstra *et al.*, 2009). Females were collected and housed using the protocol described for the field study above, with the exception

that basking opportunity and food quantity were determined by random allocation to treatment groups. The model for this experiment was a 4 x 2 factorial design with four basking opportunity treatments (3, 6, 9 and 12 hour access to the basking light) and two food quantity treatments (high and low). Basking treatments were chosen to mimic and exaggerate the yearly variation in female opportunities for basking in the field, including conditions likely to be experienced by *N. ocellatus* in extreme years (Wapstra, 2000; Wapstra *et al*., 2004; Cadby *et al.*, 2010; Cadby *et al.* 2014). More females (n = 36) were assigned to the highest (12 hour) and lowest (3 hour) basking treatments than to the intermediate (6 and 9 hour) treatments (n=28) in order to maximize the chances of detecting sex allocation responses in response to basking opportunity, which were expected to be strongest at these extremes. Half of each basking treatment group was randomly assigned to each food quantity treatment to manipulate resources available to females during gestation. Those assigned to the high food quantity treatment were provided with eight mealworms and 0.6 ml puree and protein powder mix at each feeding, whilst those assigned to the low food quantity treatment were given three mealworms and 0.3 ml puree and protein powder mix. These quantities were sufficient to provide different amounts of food whilst remaining within the boundaries for maintaining lizard health and condition. All lizards were provided with water *ad libitum* and were fed twice per week. Treatment groups were rotated among the four available wall sections of the laboratory and cage positions were randomly changed within groups once per fortnight to ensure results were not confounded by position effects. Due to several females terminating pregnancies before birth or producing non-viable offspring, which could not be sexed or measured, the final sample sizes were reduced (Supplementary Information; Table S2).

Cages were checked for neonates twice per day from the 1st December. Following birth, adult females were weighed (± 1 mg), measured (SVL ± 1 mm) and litter size was recorded. Within one day of birth offspring were weighed (± 0.1 mg), measured (SVL ± 0.01 mm), sexed, and given unique and permanent toe-clip identification before being released at the site from which their mothers were initially captured. At the completion of the majority of births within each treatment group, those individuals that had not yet given birth were checked for developing embryos by abdominal palpation. Non-pregnant females and females that had given birth were

released at their site of capture within one week of birth or identification as non-pregnant.

Laboratory study: statistical analysis

Maternal condition measures were calculated using the model developed for the long-term dataset. Maternal condition at ovulation (C_{ov}) was calculated from SVL and mass at capture and was mean-centred, whilst maternal postpartum condition (C_{pp}) was calculated from SVL and mass immediately after parturition. RLM and sex ratio were calculated in the same way as in the field study (see '*Field Study'* section above). The effect of the basking treatment, food quantity treatment, C_{ov}, and their interactions on date of birth, mean offspring mass, RLM and C_{pp} were assessed by fitting type II general linear models with basking treatment as a continuous variable (hours of basking) and food quantity treatment as a factor. To assess the effects of these variables and their interactions on sex ratio we fit a generalised linear model (GLM) with a binomial error distribution (logit link) by a type-II likelihood ratio test. Because laboratory results for the effect of temperature on offspring sex did not match those from the field study and from previous studies, we tested for an overall laboratory effect on offspring sex using an exact binomial test on the overall significance of deviation from equality of the combined sex ratio across all treatments.

All analyses were conducted in R (R Core Team, 2014) using the "lme4" package (Bates *et al.*, 2014). P-values, F-statistics and approximate denominator degrees of freedom were derived based on Kenward-Roger's approximation, using the "lmerTest" package (Kuznetsova *et al.*, 2013). In GLMM and GLM models, type-II Wald X^2 tests and likelihood ratio tests were performed using the "car" package (Fox & Weisberg, 2011).

Results

Field study

We found an overall sex ratio bias across years towards males; of the 3317 births recorded, 1757 were male and 1560 were female, a sex ratio of 0.53 (Exact binomial test; $P < 0.001$, Supplementary Information; Table S1). There was a significant correlation between mean maximum temperature during the critical sex-determining period (T_{crit}) and offspring sex (Table 1). For each 1°C increase in T_{crit} the odds of offspring being male were reduced by a factor of 0.76 (Table 1, Fig. 1). In contrast, there was no significant correlation between female condition at the onset of vitellogenesis (C_{vit}) and offspring sex, nor was there a significant interactive relationship with T_{crit} (Table 1).

Table 1: The effect of maternal condition, temperature and their interaction on litter traits in a field study of *Niveoscincus ocellatus* over the seasons 2000/01 to 2015/16.

Dependent variable	Maternal Condition (C_{vit})	Temperature	Interaction
Date of birth	$F_{(1,502.99)} = 0.65$ $P = 0.420$ $\beta = -0.460 \pm 0.566$ SE	$F_{(1,95.66)} = 108.39$ $P < 0.00001$ $\beta = -5.597 \pm 0.527$ SE	$F_{(1,148.69)} = 0.54$ $P = 0.465$ $\beta = -0.761 \pm 1.017$ SE
Mean offspring mass	$F_{(1,551.99)} = 1.96$ $P = 0.162$ $\beta = 0.005 \pm 0.004$	$F_{(1,96.55)} = 2.20$ $P = 0.142$ $\beta = 0.005 \pm 0.004$	$F_{(1,145.95)} = 0.19$ $P = 0.663$ $\beta = -0.003 \pm 0.007$
Offspring sex	$X^2 = 0.004$ $P = 0.951$ $\beta = -0.005 \pm 0.053$ SE	$X^2 = 11.236$ $P < 0.001$ $\beta = -0.273 \pm 0.081$ SE	$X^2 = 0.013$ $P = 0.908$ $\beta = 0.016 \pm 0.136$ SE
Relative litter mass (RLM)	$F_{(1,547.23)} = 11.08$ $P < 0.001$ $\beta = 0.018 \pm 0.005$ SE	$F_{(1,100.87)} = 5.92$ $P = 0.017$ $\beta = 0.013 \pm 0.005$ SE	$F_{(1,153.23)} < 0.001$ $P = 0.990$ $\beta = 0.0001 \pm 0.010$ SE
Postpartum maternal condition (C_{pp})	$F_{(1,514.44)} = 101.50$ $P < 0.0001$ $\beta = 0.376 \pm 0.037$ SE	$F_{(1, 92.72)} = 25.72$ $P < 0.0001$ $\beta = -0.187 \pm 0.036$ SE	$F_{(1, 133.33)} = 0.20$ $P = 0.654$ $\beta = -0.033 \pm 0.071$ SE

Note - Fixed effect estimates are from LMM and GLMM models. *P*-values, *F*-statistics, X^2 and β values for main effects shown are from models excluding interactions. Significant results are indicated in bold. Temperature throughout gestation (T_{gest}) is used in all models except where offspring sex is the respnse variable, in which case temperature during the critical sex determination period (T_{crit}) is used instead. Maternal condition (C_{vit}) was calculated following birth the previous year (i.e., at the beginning of vitellogenesis). β values indicate the change in the response variable for each unit change in the independent variable except where the response is Offspring sex, in which case β is the log factor change to the odds of male: female offspring for each unit change in the independent variable.

We found strong correlations (Table 1) between the mean maximum temperature experienced during gestation (T_{gest}) and both date of birth and post-partum maternal condition (C_{pp}), and a statistically significant, but minor, correlation with reproductive effort (RLM, Table 1). Specifically, for each 1°C increase in T_{gest}, birth occurred 5.60 ± 0.53 SE days earlier (Fig. 2A), C_{pp} decreased 0.19 ± 0.04 g (Fig. 2B), and RLM decreased 0.01 ± 0.005 SE. We found no correlation between T_{gest} and mean offspring mass (Table 1). C_{vit} had a statistically significant, but weak correlation with both RLM and C_{pp} (Table 1). For each 1g a mother was heavier than expected for her SVL at the beginning of vitellogenesis, RLM increased 0.018 ± 0.005 SE and C_{pp} increased 0.38 ± 0.04 SE g. There was no correlation between C_{vit} and date of birth or mean offspring mass (Table 1). Male offspring were significantly heavier than female offspring ($F_{1,2450.3} = 50.51$, $P < 0.001$), although the difference in size between the sexes was small; Mean masses of males and females were 0.539 ± 0.004 SE g and 0.529 ± 0.001 SE g, respectively. There was no interaction between T_{gest} and C_{vit} on any of the measured variables (Table 1).

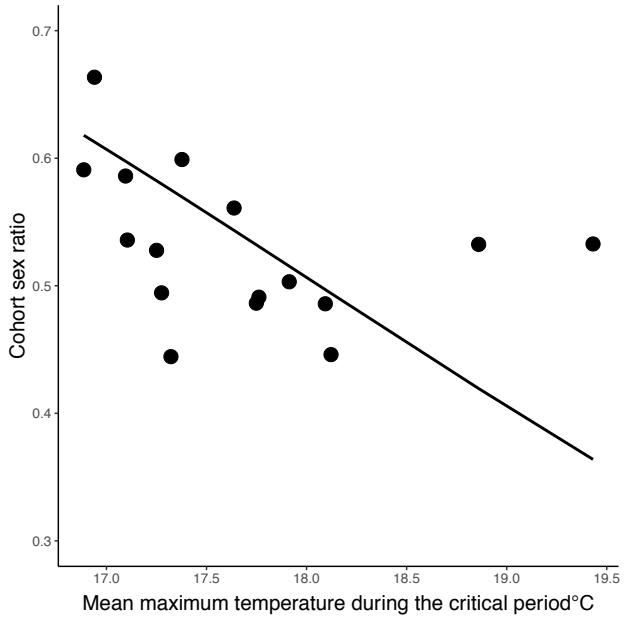

Figure 1: Mean cohort response in *Niveoscincus ocellattus* in the field of offspring sex (proportion of males) to temperature during the critical sex determining period (1 October to 14 November), for the seasons 2000/1 to 2015/6.

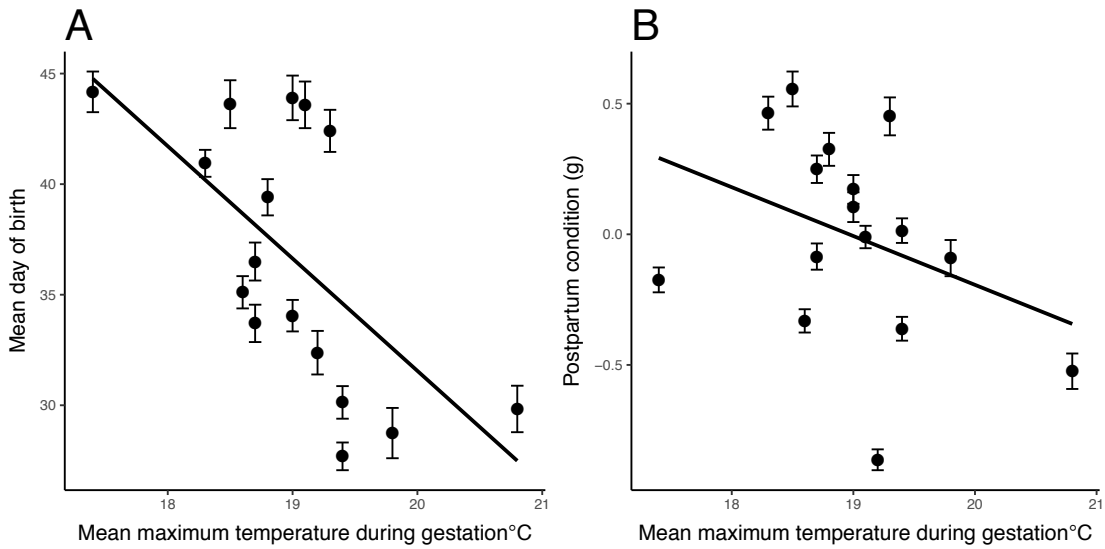

Figure 2: Mean cohort response in *Niveoscincus ocellattus* in the field of A) date of birth and B) postpartum maternal condition (C_{pp}) in response to temperature experienced during the gestation period (1 October to 31 December) for the seasons 2000/1 to 2015/6.

Experimental study

The overall sex ratio in the experimental study was male-skewed; of the 204 births recorded, 128 were males and 76 were female (Exact binomial test; $P = 0.001$; Table 2). We found no independent or interactive effects of basking treatment, food quantity treatment or maternal condition at ovulation (C_{ov}) on offspring sex or RLM (Table 3); although sex ratios showed a slight increase with increasing basking opportunity (Table 2), this effect was non-significant (Table 3). Basking treatment had a significant effect on date of birth and mean offspring mass but no significant effect on postpartum condition (Table 3). Females given more opportunity to bask produced earlier litters (-6.881 ± 0.460 SE days per hour of basking; Table 2, Supplementary Fig. S1A) consisting of larger offspring (+ 0.008 ± 0.002 SE g per hour of basking; Table 3, supplementary Fig. S1B) than females given less access to basking. Food quantity treatment had a marginally significant effect on C_{pp}, and had no significant effect on date of birth or mean offspring mass (Table 3). Females in the low food quantity treatment were in worse condition after giving birth (-0.276 ± 133 SE g) than those in the high food quantity treatment (Table 2). C_{ov} had a significant effect on C_{pp} and a marginally significant effect on mean offspring mass (Table 3). For each increase in C_{ov} of 1g, females' C_{pp} increased 0.576 ± 0.143 SE g and mean offspring mass increased 0.027 ± 0.013 SE g (Table 2).

Table 3: The effect of basking treatment (*B*), food quantity treatment (*Q*), maternal condition at ovulation (C_{ov}) and their interactions on litter characteristics and postpartum condition (C_{pp}) of *Niveoscincus ocellatus* females in a laboratory experiment.

Dependent Variable	Basking treatment (*B*)	Food quantity treatment (*Q*)	Maternal condition (C_{ov})	*B*Q* interaction	*B*C*ov interaction	*Q*C*ov interaction	*B*Q*C*ov interaction
Date of birth	**$F_{(1,84)}$= 226.64 *P* < 0.00001**	$F_{(1,84)}$ = 0.23 *P* = 0.636	$F_{(1,84)}$ = 0.45 *P* =0.504	$F_{(1,81)}$= 0.03 *P* = 0.861	$F_{(1,81)}$ = 0.14 *P* = 0.710	$F_{(1,81)}$ = 2.37 *P* = 0.128	$F_{(1,80)}$ = 2.99 *P* = 0.087
Mean offspring mass	**$F_{(1,84)}$ = 17.83 *P* < 0.0001**	$F_{(1,84)}$ = 1.59 *P* = 0.210	**$F_{(1,84)}$ = 4.07 *P* = 0.05**	$F_{(1,81)}$= 0.13 *P* = 0.719	$F_{(1,81)}$ = 2.01 *P* = 0.160	$F_{(1,81)}$ = 2.09 *P* = 0.152	$F_{(1,80)}$= 0.98 *P* = 0.325
Offspring sex	X^2 = 0.331 *P* = 0.565	X^2 = 0.0002 *P* = 0.988	X^2 = 0.007 *P* = 0.933	X^2 = 0.935 *P* = 0.331	X^2 = 0.579 *P* = 0.447	X^2 = 0.507 *P* = 0.477	X^2 = 1.526 *P* = 0.217
Relative litter mass (RLM)	$F_{(1,84)}$ = 0.45 *P* = 0.504	$F_{(1,84)}$ = 0.78 *P* = 0.381	$F_{(1,84)}$ = 0.58 *P* = 0.448	$F_{(1,81)}$= 0.06 *P* = 0.813	$F_{(1,81)}$= 0.01 *P* = 0.917	$F_{(1,81)}$ = 0.30 *P* = 0.584	$F_{(1,80)}$= 0.04 *P* = 0.844
Postpartum maternal condition (C_{pp})	$F_{(1,84)}$ = 0.97 *P* = 0.327	**$F_{(1,84)}$ = 3.82 *P* = 0.054**	**$F_{(1,84)}$ = 16.12 *P* < 0.001**	$F_{(1,81)}$= 0.002 *P* = 0.963	$F_{(1,81)}$ = 0.001 *P* = 0.972	$F_{(1,81)}$ = 0.04 *P* = 0.839	$F_{(1,80)}$= 0.20 *P* = 0.659

Note - Fixed effect estimates are from LMM and GLMM models. *P*-values, *F*-statistics and X^2 values shown are from reduced models excluding higher order interactions. Significant results are indicated in bold. Model coefficients for main effects are presented in supplemenatray table S2. Maternal condition (C_{ov}) was calculated at ovulation.

Discussion

Differential sex allocation is expected when intrinsic (e.g., body condition) or extrinsic (e.g., temperature, resource availability) factors influence variation in traits that differentially affect the fitness of male and female offspring (Charnov, 1982; Uller & Olsson, 2006; Uller *et al.*, 2007; Wapstra & Warner, 2010), or when the cost of producing offspring varies between offspring of different sexes (Fisher, 1930; Charnov, 1982). Whilst most studies consider only a single causal factor, patterns may be obscured if multiple factors influence sex allocation simultaneously. Here we examined possible interactive and independent effects of temperature and resource availability on sex allocation in *Niveoscincus ocellatus*.

We found evidence that yearly variation in sex ratios is strongly linked to annual variation in temperature during embryo development in a natural population, with the proportion of female offspring increasing in warmer years, and of male offspring in cooler years. These patterns are in accordance with our previous related

work (see Supplementary Table S3) in this system showing strong temperature effects on offspring sex ratios in the field (Wapstra *et al.*, 2004; Wapstra *et al.*, 2009; Pen *et al.*, 2010; Chapter 2: Cunningham *et al.*, 2017), and in the laboratory (Wapstra *et al.*, 2004; Pen *et al.*, 2010). Indeed, we have explicitly modelled the evolution of TSD in this population (Pen *et al.*, 2010), which explained the temperature effect as resulting from sex-specific fitness effects of date of birth, which was affected by thermal environment in both our field and laboratory studies.

In contrast, we found no evidence that female condition or resource availability at any stage during the reproductive cycle influenced sex allocation either in interaction with temperature or independently. There are several potential explanations for this lack of effects, which are related to our underlying assumptions for how resource availability might influences sex specific fitness benefits of producing males versus females. We argued that resource availability could influence offspring size at birth (*sensu* generalised Trivers & Willard, 1973), which could in turn influence offspring size at maturity, and that this may have sex-specific effects on fitness (Pen *et al.* 2010). For this to occur, however, two conditions would have to be met. First, females in good condition, or with increased access to resources, would have to divert those additional resources to their offspring rather than to their own condition or growth. We found limited support for this; whilst females in better condition at the onset of vitellogenesis did increase their reproductive effort in our field study, this effect was minor and was not replicated in the laboratory. Rather, we found that females in better condition (at both the onset of vitellogenesis, and at ovulation) and those provided with more food during gestation were in better condition after giving birth. This suggests that there may be selection on females to devote additional resources towards increasing their own condition and growth rather than towards increasing offspring size (Itonaga *et al.*, 2012a). Second, there would have to be a sex-specific link between size at birth and fitness. We have previously shown that date of birth has strong effects on offspring growth before winter and ultimately adult body size in this system, and that this has resulted in strong selection for the coupling of temperature and sex determination (Pen *et al*. 2010). For size at birth to have a similar effect, it would have to predictably translate to a larger size at the end of the activity season. Post-partum growth in *N. ocellatus* is rapid and effects influencing post-partum growth (e.g., Itonaga *et al.*, 2012a; b; Cadby *et al.*, 2014)

might offset the initial small differences in size at birth. Thus, size at birth might be a poor predictor of later size (see Qualls & Shine, 2000). Alternatively, resource availability could influence sex allocation indirectly if it affected maternal basking behaviour and, therefore affected date of birth. However, we found no effect of available resources, either alone or in interaction with thermal environment, on date of birth. The absence of an effect of resource availability on date of birth suggests that pregnant females do not alter their basking decisions according to their condition. Combined, these results suggest that there is little evidence for the effects of resource availability on the key life history traits that might provide a mechanistic link between the environment and maternal sex allocation decisions in *N. ocellatus*. Thus, selection to couple sex to resource availability may be relatively weak in this system.

One component of the study that requires additional discussion was the discordance between the strong effects of temperature on sex allocation in the field and the lack of effects of temperature observed in the laboratory, where the correlation between increasingly male-skewed litters and decreasing basking opportunity (i.e., maternal body temperature) was non-significant and sex ratios were male-skewed in all basking treatments. Discordant effects in replicated sex ratio studies are not uncommon, especially in laboratory experiments (see for example Parker, 2012; Booksmythe *et al.*, 2017). There are several possible explanations for our results. First, the birth dates invoked by our laboratory study were outside the range of birth dates that we have recorded across the 17-year period of our field study. Specifically, in our 12-hour basking treatment, the mean date of birth was earlier than all but one of those observed across the 17 years of field work, whilst birth dates in the 3 and 6 hour treatments were later than any observed over this period, corresponding to field temperatures throughout gestation of approximately 20.5^{o}C, 9.2^{o}C and 14.3^{o}C, respectively (see Figure 2A and Supplementary Figure S1A). Thus, thermal conditions in these treatments sit beyond the range of temperatures across which we have modelled the reaction norm of offspring sex response to temperature in this species, and beyond the range of temperatures they are exposed to in the wild. As thermal reaction norms with respect to temperature can have a variety of forms (e.g., MF, FM, FMF, MFM), it is possible that what we are observing is a species with a U-shaped, as opposed to a linear, reaction norm in this species (i.e., an MFM pattern, in which males are common at both high and low temperatures and females at

intermediate temperatures; e.g., Luckenbach *et al.*, 2009 in flatfishes), although this pattern has not been reported in reptiles (Quinn *et al.*, 2011). While further research is required to confirm this pattern of response, it would be consistent with the increase in male-biased litters observed in the two warmest years in our field population (Figure 1). Second, these results could have been a result of a laboratory artefact, whereby consequences of animals being kept in the laboratory (e.g., altered hormone profiles) may have impacted sex determination and thus masked any effect of temperature. For instance, laboratory-induced stress may have elevated levels of circulating corticosterone, which has been shown to have significant effects on sex determination in other reptile species (e.g., Warner *et al.*, 2009). Finally, the relatively shallow reaction norm between temperature and offspring sex in this species and the variation observed in the wild, may have meant that we did not have the power to tease out the relatively subtle effects of temperature in our laboratory experiment.

In summary, our results suggest that incorporating resource availability into our models of sex allocation decisions in *N. ocellatus* does not add additional explanatory power over and above considering temperature alone. It is, however, possible that other factors not tested in this study such as stress, age, mate quality, population dynamics, or resource availability across other temporal scales may influence sex allocation decisions in this system. For example, factors from previous years may influence reproductive effort in the current year (e.g., Doughty & Shine, 1998; Bleu *et al*., 2013) or effects may vary at different stages of the reproductive cycle. Nevertheless, none of female body condition at the beginning of vitellogenesis or at ovulation, food availability during gestation (this study), nor mating history (While & Wapstra, 2009) have been shown to be important for predicting patterns of sex allocation in *N. ocellatus*. It is, therefore, plausible that temperature is the sole factor influencing sex allocation in this species.

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References

Bates, D., Maechler, M., Bolker, B. & Walker, S. 2014. *lme4: Linear mixed-effects models using Eigen and S4*. R package version 1.1-7, http://CRAN.R-project.org/package=lme4.

Bleu, J., Le Galliard, J-F., Fitze, P., Meylan, S., Clobert, J. & Massot, M. 2013. Reproductive allocation strategies: a long-term study on proximate factors and temporal adjustments in a viviparous lizard. *Oecologia.* **171**: 141-151.

Booksmythe, I., Mautz, B., Davis, J., Nakagawa, S. & Jennions, M.D. 2017. Facultative adjustment of the offspring sex ratio and male attractiveness: a systematic review and meta-analysis. *Biol. Rev.* **92**: 108-134.

Cadby, C.D., While, G.M., Hobday, A.J., Uller, T. & Wapstra, E. 2010. Multi-scale approach to understanding climate effects on offspring size at birth and date of birth in a reptile. *Integr. Zool.* **5**: 164-175.

Cadby, C.D., Jones, S.M. & Wapstra, E. 2011. Potentially adaptive effects of maternal nutrition during gestation on offspring phenotype of a viviparous reptile. *J. Exp. Biol.* **214**: 4234-4239.

Cadby, C.D., Jones, S.M. & Wapstra, E. 2014. Geographical differences in maternal basking behaviour and offspring growth rate in a climatically widespread viviparous reptile. *J. Exp. Biol.* **217**: 1175-1179.

Cameron, E.Z. & Dalerum, F. 2009. A Trivers-Willard effect in contemporary humans: male-biased sex ratios among billionaires. *PLOS ONE.* **4**: e4195.

Charnov, E.L. 1979. Genetic evolution of patterns of sexuality - Darwinian fitness. *Am. Nat.* **113**: 465-480.

Charnov, E.L. 1982. *The Theory of Sex Allocation*. Princeton University Press, Princeton, New Jersey.

Cockburn, A., Legge, S. & Double, M.C. 2002. Sex ratio in birds and mammals: can the hypotheses be disentangled? In: *Sex Ratios Concepts and Research Methods*. (I. C. W. Hardy, ed.), pp. 266-286. Cambridge University Press, Cambridge.

Conover, D.O. 1984. Adaptive significance of temperature-dependent sex determination in a fish. *Am. Nat.* **123**: 297-313.

Cunningham, G.D., While, G.M. & Wapstra, E. 2017. Climate and sex ratio variation in a viviparous lizard. *Biol. Letters.* **13**: 20170218. (Chapter 2).

Doughty, P. & Shine, R. 1998. Reproductive energy allocation and long-term energy stores in a viviparous lizard (*Eulamprus tympanum*). *Ecology.* **79**: 1073-1083.

Fisher R. A. 1930. *The genetical theory of natural selection*. Oxford University Press, Oxford.

Fox, J. & Weisberg, S. 2011. *An {R} Companion to Applied Regression, Second Edition.* Thousand Oaks CA: Sage. http://socserv.socsci.mcmaster.ca/jfox/Books/Companion.

Frank, S.A. 1990. Sex allocation theory for birds and mammals. *Annu. Rev. Ecol. Syst.* **21**: 13-55.

Itonaga, K., Jones, S.M. & Wapstra, E. 2012a. Do gravid females become selfish? Female allocation of energy during gestation. *Physiol. Biochem. Zool.* **85**: 231-242.

Itonaga, K., Jones, S. M. & Wapstra, E. 2012b. Effects of maternal basking and food quantity during gestation provide evidence for the selective advantage of matrotrophy in a viviparous lizard. *PLOS ONE*. **7**: e41835.

Komdeur, J. 1996. Facultative sex ratio bias in the offspring of Seychelles warblers. *P. Roy. Soc. Lond. B. Bio.* **263**: 661-666.

Komdeur, J. & Pen, I. 2002. Adaptive sex allocation in birds: The complexities of linking theory and practice. *Philos. T. Roy. Soc B.* **357**: 373-380.

Kuznetsova, A., Brockhoff, P. B. & Christensen, R. H. B. 2013. lmerTest: Tests for random and fixed effects for linear mixed effect models (lmer objects of lme4 package). http://CRAN.R-project.org/package=lmerTest

Luckenbach, J. A., Borski, R. J., Daniels, H. V. & Godwin J. 2009. Sex determination in flatfishes: mechanisms and environmental influences. *Sem. Cell Dev. Biol.* **20**: 256-263.

Neaves, L., Wapstra, E., Birch, D., Girling, J.E. & Joss, J.M.P. 2006. Embryonic gonadal and sexual organ development in a small viviparous skink, *Niveoscincus ocellatus*. *J. Exp. Zool. A.* **305A**: 74-82.

Olsson, M. & Shine, R. 2001. Facultative sex allocation in snow skink lizards (*Niveoscincus microlepidotus*). *J. Exp. Biol.* **14**:120-128.

Parker, T.H. 2012. What do we really know about the signalling role of plumage colour in blue tits? A case study of impediments to progress in evolutionary biology. *Biol. Rev.* **88**: 511-536.

Pen, I., Uller, T., Feldmeyer, B., Harts, A., While, G.M. & Wapstra, E. 2010. Climate-driven population divergence in sex-determining systems. *Nature.* **468**: 436-U262.

Qualls, F.J. & Shine, R. 2000, Post-hatching environment contributes greatly to phenotypic variation between two poopulations of the Australian garden skink, *Lampropholis guichenoti*. *Biol. J. Linn. Soc.* **71**: 315-341.

Quinn, A. E., Sarre, S. D., Ezaz, T., Marshall Graves, J. A. & Georges, A. 2011. Evolutionary transitions between mechanisms of sex determinaiton in vertebrates. *Biol. Lett.* **7**: 443-448.

Radder, R.S., Pike, D.A., Quinn, A.E. & Shine, R. 2009. Offspring sex in a lizard depends on egg size. *Curr. Biol*. **19**: 1102-1105.

R Core Team. 2014. *R: A language and environment for statistical computing. R Foundation for Statistical Computing*. Vienna, Austria. http://www.R-project.org/.

Seger, J. & Stubblefield, J.W. 2002. Models of sex ratio evolution. In: *Sex ratios concepts and research methods*. (I. C. W. Hardy, ed.), pp. 2-25. Cambridge University Press, Cambridge.

Shine, R. 1980. Costs of reproduction in reptiles. *Oecologia.* **46**: 92-100.

Thompson, M.B., Speake, B.K., Stewart, J.R., Russell, K.J. & McCartney, R.J. 2001. Placental nutrition in the Tasmanian skink, *Niveoscincus ocellatus*. *J. Comp. Physiol. B.* **171**: 155-160.

Trivers, R.L. & Willard, D.E. 1973. Natural selection of parental ability to vary the sex-ratio of offspring. *Science* **179**: 90-92.

Uller, T. & Olsson, M. 2006. No seasonal sex-ratio shift despite sex-specific fitness returns of hatching date in a lizard with genotypic sex determination. *Evolution.* **60**: 2131-2136.

Uller, T., Pen, I., Wapstra, E., Beukeboom, L.W. & Komdeur, J. 2007. The evolution of sex ratios and sex-determining systems. *Trends Ecol. Evol.* **22**: 292-297.

Uller, T., While, G.M., Cadby, C.D., Harts, A., O'Connor, K., Pen, I. & Wapstra, E. 2011. Altitudinal divergence in maternal thermoregulatory behaviour may be driven by differences in selection on offspring survival in a viviparous lizard. *Evolution.* **65**: 2313-2324.

Uller, T. & While, G. M. 2014. Evaluation of offspring size-number invariants in 12 species of lizard. In: *Reproductive biology and phylogeny of lizards and tuatara*. (J. L. Rheubert, D. S. Siegel & S. E. Trauth, eds), pp 425-447. CRC Press, Boca Raton, Florida.

Wapstra, E., Swain, R., Jones, S.M. & O'Reilly, J. 1999. Geographic and annual variation in reproductive cycles in the Tasmanian spotted snow skink, *Niveoscincus ocellatus* (Squamata: Scincidae). *Aust. J. Zool.* **47**: 539-550.

Wapstra, E. 2000. Maternal basking opportunity affects juvenile phenotype in a viviparous lizard. *Funct. Ecol.* **14**: 345-352.

Wapstra, E. & O'Reilly, J.M. 2001. Potential 'costs of reproduction' in a skink: Inter- and intrapopulational variation. *Austral Ecol*. **26**: 179-186.

Wapstra, E. & Swain, R. 2001. Geographic and annual variation in life-history traits in a temperate zone Australian skink. *J. Herpetol*. **35**: 194-203.

Wapstra, E., Swain, R. & O'Reilly, J.M. 2001. Geographic variation in age and size at maturity in a small Australian viviparous skink. *Copeia.* **3**: 646-655.

Wapstra, E., Olsson, M., Shine, R., Edwards, A., Swain, R. & Joss, J.M.P. 2004. Maternal basking behaviour determines offspring sex in a viviparous reptile. *P. Roy. Soc. Lond. B. Bio.* **271**: S230-S232.

Wapstra, E., Uller, T., Sinn, D.L., Olsson, M., Mazurek, K., Joss, J. & Shine, R. 2009. Climate effects on offspring sex ratio in a viviparous lizard. *J. Anim. Ecol.* **78**: 84-90.

Wapstra, E., Uller, T., While, G.M., Olsson, M. & Shine, R. 2010. Giving offspring a head start in life: field and experimental evidence for selection on maternal basking behaviour in lizards. *J. Evol. Biol.* **23**: 651-657.

Wapstra, E. & Warner, D.A. 2010. Sex allocation and sex determination in squamate reptiles. *Sex. Dev.* **4**: 110-118.

Warner, D.A. & Shine, R. 2008. The adaptive significance of temperature-dependent sex determination in a reptile. *Nature.* **451**: 566-568.

Warner, D.A., Radder, R.S. & Shine, R. 2009. Exposure during embryonic development affects offspring growth and sex ratios in opposing directions in two lizard species with environmental sex determination. *Phisiol. Biochem. Zool.* **82**: 363-371.

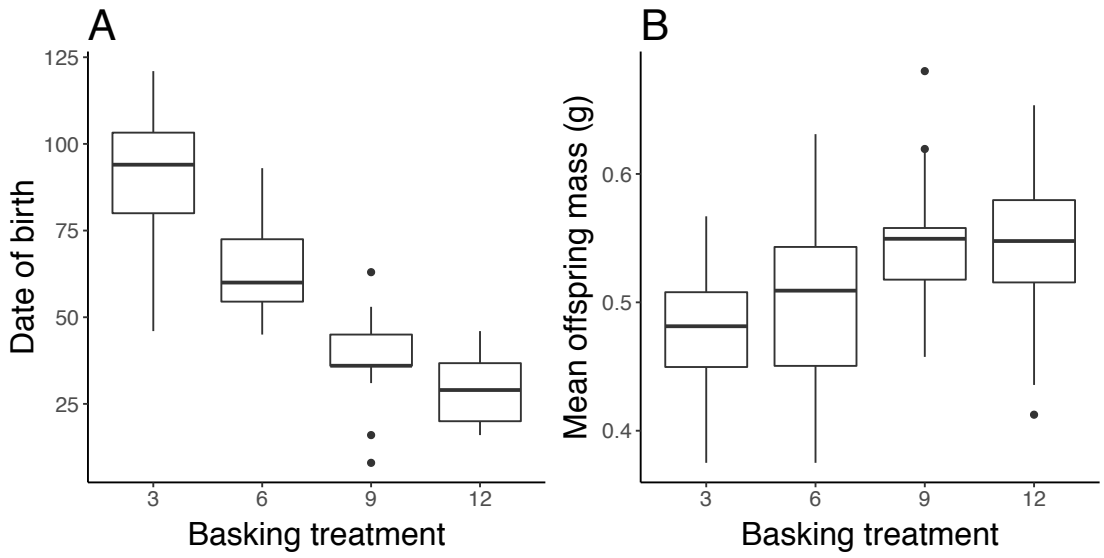
West, S.A. 2009. *Sex Allocation*. Princeton University Press, Oxford.

While, G.M. & Wapstra, E. 2009. Snow skinks (*Niveoscinus ocellatus*) do not shift their sex allocation patterns in response to mating history. *Behaviour*. **146**: 1405-1422.

Chapter 3: Supplementary Information

Table S1: Data collected in a field study across the seasons 2000/1 to 2015/6.

Year	Number of litters	C_{vit} measures	Total offspring	Total males	Sex ratio
2000-2001	87	0	189	84	0.44
2001-2002	98	26	220	130	0.59
2002-2003	90	36	205	115	0.56
2003-2004	104	35	211	140	0.66
2004-2005	91	46	215	126	0.59
2005-2006	108	46	278	124	0.45
2006-2007	91	44	220	107	0.49
2007-2008	66	35	159	80	0.50
2008-2009	91	39	231	123	0.53
2009-2010	96	44	237	127	0.54
2010-2011	103	42	252	133	0.53
2011-2012	90	50	224	110	0.49
2012-2013	83	36	180	89	0.49
2013-2014	78	37	197	118	0.60
2014-2015	71	35	177	86	0.49
2015-2016	48	25	122	65	0.53
Total	1395	576	3317	1757	0.53



Supplementary Figure S1: Effect of basking treatment on A) date of birth and B) mean offspring mass in *Niveoscincus ocellattus* in a laboratory experiment.

Table S2: Main effect model coefficients from generalised (logit) linear model (Sex ratio) and general linear models (remaining variables) in a 4 x 2 factorial laboratory experiment with four basking treatments (3, 6, 9 and 12-hours) and two food quantity treatments (high and low) in *Niveoscincus ocellatus.*

Dependent Variable	Basking treatment (*B*)	Food quantity treatment (*Q*)	Maternal condition (C_{ov})	*B*Q*	*B*C*$_{ov}$	*Q*C*$_{ov}$	*B*Q*C*$_{ov}$
Date of birth	*Int* = 106.95 ± 4.30 SE *β* = -6.881 ± 0.460 SE	*Int* = 106.95 ± 4.30 SE *β* = 0.454 ± 3.036 SE	*Int* = 106.95 ± 4.30 SE *β* = -2.19 ± 3.273 SE	*Int* = 105.5 ± 5.7 SE *β* = -0.24 ± 0.93 SE	*Int* = 105.5 ± 5.7 SE *β* = -0.21 ± 1.09 SE	*Int* = 105.5 ± 5.7 SE *β* = 10.26 ± 6.66 SE	*Int* = 104 .4 ± 5.7 SE *β* = -3.86± 2.23 SE
Mean offspring mass	*Int* = 0 .470 ± 0.017 SE *β* = 0.008 ± 0.002 SE	*Int* = 0 .470 ± 0.017 SE *β* = -0.015 ± 0.012 SE	*Int* = 0 .470 ± 0.017 SE *β* = 0.026 ± 0.013 SE	*Int* = 0 .465 ± 0.023 SE *β* = -0.001 ± 0.004 SE	*Int* = 0 .465 ± 0.023 SE *β* = -0.005 ± 0.004 SE	*Int* = 0 .465 ± 0.023 SE *β* = 0.038 ± 0.027 SE	*Int* = 0 .468 ± 0.023 SE *β* = 0.009 ± 0.009 SE
Offspring sex	*Int* = 0.745 ± 0.414 SE *β* = -0.026 ± 0.045 SE	*Int* = 0.745 ± 0.414 SE *β* = -0.005 ± 0.292 SE	*Int* = 0.745 ± 0.414 SE *β* = -0.025 ± 0.295 SE	*Int* = 1.081 ± 0.538 SE *β* = 0.089 ± 0.092 SE	*Int* = 1.081 ± 0.538 SE *β* = 0.078 ± 0.103 SE	*Int* = 1.081 ± 0.538 SE *β* = -0.443 ± 0.623 SE	*Int* = 1.103 ± 0.541 SE *β* = 0.278 ± 0.226 SE
Relative litter mass (RLM)	*Int* = 0 .253 ± 0.022 SE *β* = -0.002 ± 0.002 SE	*Int* = 0 .253 ± 0.022 SE *β* = -0.014 ± 0.016 SE	*Int* = 0 .253 ± 0.022 SE *β* = 0.013 ± 0.017 SE	*Int* = 0 .259 ± 0.030 SE *β* = 0.001 ± 0.005 SE	*Int* = 0 .259 ± 0.030 SE *β* = 0.0002 ± 0.006 SE	*Int* = 0 .259 ± 0.030 SE *β* = -0.019 ± 0.035 SE	*Int* = 0 .260 ± 0.030 SE *β* = 0.002 ± 0.012 SE
Postpartum maternal condition (C_{pp})	*Int* = 0.75 ± 0.188 SE *β* = -0.027 ± 0.020 SE	*Int* = 0.75 ± 0.188 SE *β* = -0.276 ± 0.133 SE	*Int* = 0.75 ± 0.188 SE *β* = 0.576 ± 0.143 SE	*Int* = 0.74 ± 0.26 SE *β* = -0.001 ± 0.042 SE	*Int* = 0.74 ± 0.26 SE *β* = 0.0005 ± 0.05 SE	*Int* = 0.74 ± 0.26 SE *β* = -0.06 ± 0.30 SE	*Int* = 0.76 ± 0.26 SE *β* = -2.19 ± 3.273 SE

Note - Coefficients presented are from reduced models excluding higher-order interactions. Significant effects are shown in bold (see tables 2 & 3 in main manuscript). Maternal condition (C_{ov}) was calculated at ovulation. *β* values indicate the change in the response variable for each unit change in the independent variable for basking treatment and maternal condition, except where the response is Offspring sex, in which case *β* is the log factor change to the odds of male: female offspring for each unit change in the independent variable. *β* values for food quantity treatments indicate the difference between the high (intercept) and low treatments.

Table S3: Summary of previously reported studies demonstrating a relationship between environmental temperature during the period of sex determination and offspring sex and between date of birth and offspring sex. Note that data from previously reported observational field studies are included within the current dataset and are, thus, not independent results.

Predictor	Experimental units	Sex ratio	Statistics	Study type	Reference
Basking (2 treatments)	4-hour (N = 45) 10-hour (N = 27) Between groups	0.69 0.33	P_{bin} = 0.016 P_{bin} = 0.12 G = 8.75, P < 0.0015	Experimental	Wapstra *et al.*, 2004; Pen *et al.*, 2010
Date of birth*	1 (N = 13) 2 (N = 16) 3 (N = 28) 4 (N = 17)	0.38, 0.45 0.55, 0.70	r^2_{model} = 0.0870 X^2 = 5.6319, P = 0.0176	Observational field (2000/1)	Wapstra *et al.*, 2004
Temperature	By litter By cohort (N = 7)	0.57 odds per +1°C -0.16 / °C†	$F_{(1,675)}$ = 21.81, P < 0.001 $F_{(1,6)}$ = 14.14, R^2 = 0.738, P = 0.013	Observational field (2000/1 – 2006/7)	Wapstra *et al.*, 2009; Pen *et al.*, 2010
Date of birth	By cohort (N = 7)	0.01 / day†	$F_{(1,6)}$ = 9.87, R^2 = 0.67, P = 0.024	Observational field (2000/1 – 2006/7)	Wapstra *et al.*, 2009; Pen *et al.*, 2010
Temperature	By litter	0.869 odds per +1°C	X^2 = 6.392, P = 0.011	Observational field (2000/1 – 2015/16)	Cunningham *et al.* 2017 (chapter 2)

* Sex ratios presented in four 9-day increments and estimated from plots, Statistics from logistic model of continuous data.
† Linear slope of sex ratio relationship estimated from plots.

Chapter 3, Appendix:

Linear mixed models (Models testing the effect of environmental temperature on date of birth, mean offspring mass, relative litter mass (RLM), postpartum maternal condition (C_{pp}) and offspring sex, corresponding to the analysis presented in Table 2 in chapter 3, but fit by maximum likelihood and with year included as a factorial random effect. Note, that significance of the effect of temperature on RLM and C_{pp} is non-significant in these tests, differing from the statistical significance of these effects presented in the main text and the published paper (Gruber *et al.*, 2018). However, because all individuals within each site had the same measure for temperature within years, temperature and year were inescapably confounded in site-specific models. Estimates of the effect (βs) of temperature on both RLM and C_{pp} did not differ substantially from estimates in the models fit without year as a random effect presented in the main text. Thus, although the statistical significance differed when year was included as a random effect, this is likely a consequence of confounding between year and temperature. Consequently the analysis presented in this chapter should be accepted as a better test of the significance of temperature on date of birth and offspring sex. Additionally, discussion of the effect of temperature on these factors did not contribute to the conclusions presented in this chapter and do not, therefore, alter our interpretation of the results of this study.

Table A1: The effect of maternal condition, temperature and their interaction on litter traits in a field study of *Niveoscincus ocellatus* over the seasons 2000/01 to 2015/16.

Dependent variable	Maternal Condition (C_{vit})	Temperature	Interaction
Date of birth	$F_{(1,496.61)} = 0.03$ $P = 0.856$ $\beta = 0.102 \pm 0.560$ SE	$\mathbf{F_{(1,15.37)} = 10.15}$ $\mathbf{P = 0.006}$ $\mathbf{\beta = -5.205 \pm 1.634}$ **SE**	$F_{(1,407.7)} = 0.45$ $P = 0.501$ $\beta = -0.589 \pm 0.875$ SE
	$\sigma_{ID} = 40.02 \pm 6.33$ $\sigma_{IDxTemp} = 0.47 \pm 0.68$ $\sigma_{Year} = 19.38 \pm 4.40$ $\sigma_{resid} = 25.45 \pm 5.04$		$\sigma_{ID} = 40.23 \pm 6.34$ SD $\sigma_{IDxTemp} = 0.48 \pm 0.69$ SD $\sigma_{Year} = 19.41 \pm 4.41$ SD $\sigma_{resid} = 25.35 \pm 5.03$ SD
Mean offspring mass	$F_{(1,398.1)} = 0.74$ $P = 0.389$ $\beta = 0.004 \pm 0.004$ SE	$F_{(1,20.1)} = 0.68$ $P = 0.420$ $\beta = 0.005 \pm 0.006$ SE	$F_{(1,433.3)} = 0.44$ $P = 0.506$ $\beta = -0.005 \pm 0.007$ SE
	$\sigma_{ID} = 0.00 \pm 0.03$ SD $\sigma_{IDxTemp} = 0.00 \pm 0.01$ SD $\sigma_{Year} = 0.00 \pm 0.01$ SD $\sigma_{resid} = 0.00 \pm 0.05$ SD		$\sigma_{ID} = 0.00 \pm 0.03$ SD $\sigma_{IDxTemp} = 0.00 \pm 0.01$ SD $\sigma_{Year} = 0.00 \pm 0.01$ SD $\sigma_{resid} = 0.00 \pm 0.04$ SD
Offspring sex	$X^2_{(1,576)} = 0.01$ $P = 0.921$ $\beta = -0.009 \pm 0.087$ SE	$\mathbf{X^2_{(1,576)} = 10.738}$ $\mathbf{P = 0.001}$ $\mathbf{\beta = -0.273 \pm 0.083}$ **SE**	$X^2_{(1,576)} = 0.008$ $P = 0.930$ $\beta = 0.012 \pm 0.139$ SE
	$\sigma_{ID} = 0.00 \pm 0.00$ SD $\sigma_{IDxTemp} = 0.00 \pm 0.00$ SD $\sigma_{Year} = 0.00 \pm 0.04$ SD		$\sigma_{ID} = 0.00 \pm 0.00$ SD $\sigma_{IDxTemp} = 0.00 \pm 0.00$ SD $\sigma_{Year} = 0.00 \pm 0.04$ SD
Relative litter mass (RLM)	$\mathbf{F_{(1,494.9)} = 8.48}$ $\mathbf{P = 0.004}$ $\mathbf{\beta = 0.012 \pm 0.01}$ **SE**	$F_{(1,17.2)} = 1.37$ $P = 0.258$ $\beta = 0.012 \pm 0.01$ SE	$F_{(1,394.4)} = 0.03$ $P = 0.857$ $\beta = 0.002 \pm 0.01$ SE
	$\sigma_{ID} = 0.00 \pm 0.04$ SD $\sigma_{IDxTemp} = 0.00 \pm 0.01$ SD $\sigma_{Year} = 0.00 \pm 0.02$ SD $\sigma_{resid} = 0.00 \pm 0.06$ SD		$\sigma_{ID} = 0.00 \pm 0.04$ SD $\sigma_{IDxTemp} = 0.00 \pm 0.01$ SD $\sigma_{Year} = 0.00 \pm 0.02$ SD $\sigma_{resid} = 0.00 \pm 0.06$ SD
Postpartum maternal condition (C_{pp})	$\mathbf{F_{(1,552.5)} = 49.34}$ $\mathbf{P < 0.0001}$ $\mathbf{\beta = 0.259 \pm 0.037}$ **SE**	$F_{(1,15.28)} = 2.28$ $P = 0.151$ $\beta = -0.183 \pm 0.121$ SE	$F_{(1,311.5)} = 0.47$ $P = 0.493$ $\beta = -0.042 \pm 0.062$ SE
	$\sigma_{ID} = 0.02 \pm 0.15$ SD $\sigma_{IDxTemp} = 0.00 \pm 0.04$ SD $\sigma_{Year} = 0.11 \pm 0.33$ SD $\sigma_{resid} = 0.17 \pm 0.42$ SD		$\sigma_{ID} = 0.03 \pm 0.16$ SD $\sigma_{IDxTemp} = 0.00 \pm 0.05$ SD $\sigma_{Year} = 0.11 \pm 0.33$ SD $\sigma_{resid} = 0.17 \pm 0.42$ SD

Note - Fixed effect estimates are from LMM and GLMM models. *P*-values, *F*-statistics, X^2 and β values for main effects shown are from models excluding interactions. Significant results are indicated in bold. Temperature throughout gestation (T_{gest}) is used in all models (i.e. LMM models) except where offspring sex is the respnse variable (i.e., GLMM model), in which case temperature during the critical sex determination period (T_{crit}) is used instead. Maternal condition (C_{vit}) was calculated following birth the previous year (i.e., at the beginning of vitellogenesis). β values indicate the change in the response variable for each unit change in the independent variable except where the response is Offspring sex, in which case β is the log factor change to the odds of male: female offspring for each unit change in the independent variable.

Chapter 4:

Plastic Rates of Development and the Effect of Thermal Extremes on Offspring Fitness in a Cold-Climate Viviparous Lizard

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Abstract

Populations at the climatic margins of a species' distribution can be exposed to conditions that cause developmental stress, resulting in developmental abnormalities. Even within the thermal range of normal development, phenotypes often vary with developmental temperature (i.e. thermal phenotypic plasticity). These effects can have significant consequences for organismal fitness and, thus, population persistence. Reptiles, as ectotherms, are particularly vulnerable to thermal effects on development and are, therefore, considered to be at comparatively high risk from changing climates. Understanding the extent and direction of thermal effects on phenotypes and their fitness consequences is crucial if we are to make meaningful predictions of how populations and species will respond as climates warm. Here, we experimentally manipulated the thermal conditions experienced by females from a high-altitude cold-adapted population of the viviparous skink, *Niveoscincus ocellatus*, to examine the consequences of thermal conditions at the margins of this population's normal temperature range. We found strong effects of thermal conditions on the development of key phenotypic traits that have implications for fitness. Specifically, we found that offspring born earlier as a result of high temperatures during gestation had increased growth over the first winter of life, but there was no effect on offspring survival, nor was there an effect of developmental temperature on the incidence of developmental abnormalities. Combined, our results suggest that advancing birth dates that result from warming climates may have positive effects in this population via increased growth.

Introduction

Temperature can have profound effects on the developmental trajectory of organisms (i.e., thermal developmental plasticity). These effects have been particularly well studied in ectotherms, in which the thermal environment an embryo experiences during development regulates key physiological processes and, through this, a range of phenotypic traits including development rate, morphology, behaviour, performance, physiology and even sex (Elphick & Harlow, 1997; Pearson & Warner, 2016; Chapter 2: Cunningham, While, & Wapstra, 2017; Shine, Noble, Stenhouse, & Schwanz, 2018; While *et al*., in press). Importantly, these effects can have significant consequences for fitness both in the short and the long term (Caley & Schwarzkopf, 2004; Noble *et al.*, 2018) and can, therefore, affect key evolutionary and ecological processes (Uller, 2008; Moczeck *et al.*, 2011).

The shape and nature of developmental responses to temperature can be conceptualised in terms of thermal reaction norms (*sensu* Scheiner, 1993; Georges, Beggs, Young, & Doody, 2005). Importantly, reaction norms may differ among populations as the result of physiological constraints on optimal development across developmental temperatures and/or local adaptation to optimise phenotypes across temperatures within the range that populations have experienced historically (Caley and Schwarzkopf, 2004; Visser, 2008; Chevin, Lande, & Mace, 2010; Merilä, 2012). Such population-specific reaction norms can help us understand how micro and macroclimates affect phenotypic traits related to fitness within populations (e.g., Monasterio, Shoo, Salvador, Iraeta, & Díaz, 2013; While *et al.*, 2015). Unsurprisingly, this information is increasingly being used to predict the potential impact of projected changes in climate on population and species persistence via effects on the development of fitness related traits (Noble *et al.*, 2018). However, as climates change, populations may increasingly experience environmental conditions that are at, or beyond, the range of historical temperatures and, therefore, of characterised reaction norms. This is particularly pertinent for populations at the margins of a species' climatic range. Thus, a key challenge for evolutionary ecologists is to understand how such extreme conditions affect phenotypic traits that are related to fitness.

Exposure to novel environmental conditions may impact organismal fitness in a variety of ways. Local adaptation of thermal reaction norms may be limited by physiological constraints and gene flow from other populations. Thus, populations close to or at a species' thermal limits are more likely to be exposed to conditions that disrupt normal development, resulting in developmental stress. Phenotypically, this can manifest as increased incidence of embryonic mortality and/or abnormalities (Yntema, 1960; Löwenborg, Shine, & Hagman, 2011; Telemeco, Warner, Reida, & Janzen, 2013). Even where populations do not experience conditions that disrupt normal development, exposure to historically extreme temperatures may have fitness effects via thermally plastic phenotypes. For example, warming climates have resulted in the early onset of breeding in many ectotherms (While & Uller, 2014; Cohen, Lajeunesse, & Rohr, 2018), which can have positive effects on population persistence and growth (e.g., McCaffery & Maxwell, 2010; Phillimore, Stålhandske, Smithers, & Bernard, 2012). These positive effects may be relatively widespread, especially for populations that currently occur at the lower end of their species' thermal limits. Conversely, changes in phenology due to rising temperatures, can affect interactions with the biotic and abiotic environment, resulting in trophic mismatches leading to population decline (Visser & Both, 2005; Willette, Tucker, & Janzen, 2005; Noble *et al.*, 2018).

Despite the fundamental importance of addressing the consequences of altered thermal conditions for phenotypic development, we currently have a limited understanding of the links between the proximate effects of climate on individuals, the processes by which these effects occur and their consequences for population persistence. There are two main reasons for this. First, population-specific thermal reaction norms of development are relatively poorly characterised, particularly for ectothermic vertebrates such as reptiles (While *et al.*, in press). To address this, we must characterise developmental plasticity across (and beyond) the range of temperatures currently experienced by populations in the wild, especially those located at a species' climatic limits. Second, we need to integrate this characterisation of thermal plasticity with longitudinal studies of these populations that examine the long-term fitness consequences of resultant phenotypes. While recent meta-analytical work has shown that phenotypic effects in response to early thermal environments can have long-lasting effects in a number of reptile species (Noble *et al.*, 2018), long-term

studies directly assessing the covariance between traits and fitness are extremely rare (but see Warner & Shine, 2008; Wapstra, Uller, While, Olsson, & Shine, 2010). Addressing these shortcomings will give us a far greater ability to make accurate predictions regarding the consequences that exposure to novel developmental conditions (for example via climate change) will have for reptiles.

Here we addressed these shortcomings, using a long-term study of an alpine population of the viviparous lizard, *Niveoscincus ocellatus*, for which we have a well characterised thermal reaction norm for phenology (Chapter 2: Cunningham *et al.*, 2017). This enabled us to develop two experimental thermal treatments designed to expose females to conditions at the extremes of the characterised thermal reaction norm. To explore the effects of the different thermal environments during development on offspring growth and survival we performed a mark-recapture study comparing offspring from these treatments with those from a field cohort. We predicted that exposure to thermal conditions at the extremes of the population's characterised thermal reaction norm would have a number of potential outcomes. First, if the thermal conditions experienced by females represent conditions that induce developmental stress (i.e., beyond the population's thermal limits), then offspring would show increased incidences of developmental abnormalities and/or embryonic mortality, reduced survival and/or growth. Alternatively, if the thermal conditions experienced by a female do not compromise development (i.e., they fall within the population's thermal limits), then we predicted that offspring size at birth would be affected by thermal treatment and that birth dates would advance with increasing temperature. Advances in date of birth can have either positive or negative outcomes. Earlier birth dates may have positive effects on offspring growth and survival during the first year of life due to an extended active period before the onset of winter, with the opposite being true when thermal conditions result in later dates of birth (see Wapstra, Swain, & O'Reilly, 2001; Wapstra *et al.*, 2010). Conversely, exposure to thermal conditions at the extremes of the characterised thermal reaction norms may impact fitness negatively if shifts in date of birth, or other phenotypic effects of temperature, alter the way in which an organism interacts with its environment. For example, if food availability varies temporally (for example, see Wapstra & Swain, 1996 for seasonal variation in prey availability for this species), then date of birth may affect offspring survival and growth.

Materials and Methods

Study species and study site

Niveoscincus ocellatus is a small (3–10 g, 60-75 mm snout-vent-length (SVL)) viviparous lizard endemic to Tasmania, Australia with a wide altitudinal distribution from sea level to 1200 m (Uller *et al.*, 2011; Cadby, Jones, & Wapstra, 2014). Populations across the species' range exhibit a degree of genetic isolation (Cliff, Wapstra, & Burridge, 2015) as well as variation across climates in a number of traits including: thermoregulatory behaviour (Uller *et al.*, 2011; Cadby *et al.*, 2014), size at maturity (Wapstra *et al.*, 2001), size at birth (Wapstra & Swain, 2001), litter size (Wapstra & Swain, 2001; Pen *et al.*, 2010) and sex determination system (Pen *et al.,* 2010; Chapter 2: Cunningham *et al.*, 2017; Hill, Ezaz, Wapstra, & Burridge, in press). This study was conducted in a population at the climatic and altitudinal extreme of the species' distribution at Lake Augusta on the Central Plateau of Tasmania (41^{o}86' S, 147^{o}53' E; elevation 1150 m) which has been extensively studied since the 1990s. In this population, lizards are active from September to April, dependent on environmental temperatures (Wapstra *et al.*, 1999). Females mature at 2-4 years, produce an annual litter of 1-8 offspring and live up to 12 years. Mating occurs in autumn and females store sperm over winter when animals are inactive (Jones, Wapstra, & Swain, 1997; Wapstra *et al.*, 1999). Ovulation is highly synchronised, occurring in mid-October, and is consistent among years (Wapstra *et al.*, 1999). Development rates and birth dates correlate with environmental temperatures experienced during gestation, such that births occur earlier in warm years and later in cold years (Uller *et al.*, 2011; Chapter 2: Cunningham *et al.*, 2017; see Fig. 1).

Experimental protocol

We combined data from a 16 year field study of this population, in which individuals are recaptured each year to measure female reproductive traits and offspring traits at birth, with an experimental manipulation of the thermal environment designed to mimic conditions at the extreme ends of the natural population's characterised thermal reaction norm. We compared traits measured at birth and following the first

winter of offspring from two experimental treatment groups and those from the field cohort of the long-term study population from the same year as the experiment to examine the extent to which the thermal environment an offspring experiences during development affects fitness related traits.

i) *Field Study*

Between the Austral summer seasons of 2000/2001 and 2015/2016, we monitored female traits and offspring traits at birth at the Lake Augusta site (see Chapter 2: Cunningham *et al.*, 2017). Each year approximately 100 pregnant females (~95% of the population) were captured at the end of gestation (January) using mealworm 'fishing' (Wapstra *et al.*, 1999) and noosing techniques and brought back to the terrestrial ecology facilities at the University of Tasmania. Here, females were housed individually in plastic terraria (30 x 20 x 15 cm) with a wooden cover for shelter, a rock for basking, and paper pellets as a substrate. All lizards were fed three times each week with live insects (*Tenebrio* larvae) and crushed fruit (Heinz baby food) with added protein powder (Nature's Way) and were provided with water *ad libitum*. Natural day/night cycles were simulated using overhead lights (14:10 light dark photoperiod) and each terrarium was fitted with a basking light that was available for 8 hours per day to provide a thermal gradient for basking from 16 to 40 °C.

Females remained under these conditions until birth, at which point offspring were removed from their mother, measured (SVL, ± 0.01 mm and mass, ± 0.1 mg), sexed by hemipene eversion, and toe-clipped for future identification. In the year of this study, we also measured sprint speed at birth as an indictor of whole-body performance. Prior to sprint trials each lizard was placed in a sealed metal chamber immersed for a minimum of 20 minutes in a water bath held at 28 ± 0.1° to ensure that all animals had equilibrated to the average active body temperature maintained in the field (Wapstra, 2000) and the temperature at which sprint speed is maximized for this species. Lizards were then encouraged to sprint along a 100 x 10 cm track, heated to 28 ± 0.1 °C by gently tapping them on the tail from behind with an artist's paintbrush. Time taken for offspring to run each section of 25 cm was recorded by evenly spaced infrared sensors. Trials were performed twice and the quickest time over 25 cm across both trials was used as a measure of optimum performance. Lizards

assessed as not running effectively in response to the encouragement (or ran the wrong way, stopped or turned around) were excluded. Following collection of all offspring data, mothers were released at one of 12 pre-designated release locations within the study site closest to their capture location and offspring were released randomly at one of these locations (to avoid confounding maternal sites and fitness with offspring sites and fitness – see Wapstra *et al.*, 2010).

Although we use all 16 years of data to characterise thermal reaction norms in this population, the focus of this study was on the 2001/2002 cohort ("Field cohort", hereafter), which corresponded with the year of the experimental study (see below). In this year of the long-term study 108 females gave birth to a total of 432 offspring, of which 18 were non-viable.

ii) Experimental Study

We combined data from the above field study with an experimental manipulation carried out in the Austral summer of 2001/2002. For this experiment we collected an additional 69 pregnant females soon after ovulation (Wapstra *et al.*, 1999) from a population next to the long-term field study site. Lizards were captured and housed in the same way as described for the field study above, except that, throughout gestation, females were randomly allocated to one of two basking regimes in which basking lights were turned on for either 4 or 10 hours per day (N = 39 and 30 for the 10- and 4-hour treatments, respectively). These basking treatments were designed to mimic environmental temperatures at the higher and lower end of those experienced by the natural population (see Fig. 2). Terraria were positioned randomly in the laboratory and repositioned fortnightly to minimize positional effects. Towards the end of gestation, terraria were checked twice daily for neonates. At birth offspring traits were recorded in an identical way to those described for the natural population (see above). Mothers were then released at their site of capture and offspring were released randomly at one of the 12 release locations within the long-term study site (see *field study*, above). Thus, treatment animals and animals from the field cohort experienced the same conditions over winter. Final sample sizes for the treatment groups were 107 offspring from 33 females and 83 offspring from 25 females from the 10- and 4- hour treatments, respectively. These numbers were slightly reduced from those initially

captured because some females were non-pregnant and some died during the experiment.

Estimates of offspring fitness

To examine the consequences of thermal conditions during development for offspring fitness (measured as offspring growth and survival) we recaptured all surviving offspring from the treatment groups and from the field cohort the following spring (October-November, 2002), after the winter hibernation. Upon recapture, offspring were measured for mass and SVL and were then marked with silver non-toxic marker (Artline® calligraphy pen) before being released at their site of capture. Offspring recaptures took place until only marked offspring were observed over several days of survey (~4 weeks). As average juvenile dispersal over the first year is 17.84 m ± 2.73 (Atkins, Swain, Wapstra, & Jones, 2007) and the site is located on a dam wall and is flanked on one side by water and, on the other, by unsuitable habitat, it is unlikely that any individuals moved outside the boundaries of the field site. Thus, estimates of offspring survival are unlikely to be compromised by dispersal.

Statistical analysis

We first quantified the thermal reaction norm of dates of birth for the population by fitting a linear model of litter birth dates across all years of the 16-year long-term dataset against the mean-maximum daytime temperatures during the gestation period (October 15 – January 14; see Uller *et al.*, 2011; Chapter 2: Cunningham *et al.*, 2017) at the long-term study site. Temperature data were obtained via the Australian Bureau of Meteorology website, from a station close to the study site at Liawenee (41°53’58’’S, 146°40’05’’E). We then compared the mean date of birth of the treatment groups to this fitted model to estimate the equivalent temperature experienced during gestation of the two laboratory treatments. To test that the effect of basking treatment affected litter dates of birth among the three study groups (field cohort, 4 hour basking treatment and 10 hour basking treatment), as expected, we fit a linear model (LM) with litter birth date as the dependent variable and group as a fixed factor. This analysis confirmed that dates of birth differed substantially between

groups (see results), confirming that the thermal environment affected developmental rates. We also tested whether there were differences between groups in a) the likelihood of litters including one or more non-viable offspring, b) offspring size (SVL) at birth, and c) offspring performance (sprint speed). To test for differences in the probability of litters containing non-viable offspring we fit a type II generalised linear model (GLM) with binomial distribution (logit link). To test whether size (SVL) or performance (sprint speed) of offspring differed between groups we fit linear mixed models (LMMs), including litter ID as a random effect to control for similarities among siblings. In the model testing for differences in sprint speed we also included SVL as a covariate.

We then examined the effects of differences between study groups in development on offspring growth and survival. To test for effects on offspring survival, we fit a generalised linear mixed model (GLMM) with binomial distribution (logit link) with offspring survival (i.e., whether offspring were recaptured) as the dependent variable, birth date, group and their interaction as fixed factors, and SVL at birth as a covariate to account for differences in size between individuals at birth (see results). As above, we included litter ID as a random effect. To test for effects on offspring growth we fit an LMM, with offspring size at recapture as the dependent variable, date of birth, group and their interaction as fixed factors, SVL at birth and recapture date, and litter ID as a random effect. Because only recaptured offspring were included in this model, sample sizes were reduced (see results). In all models, continuous independent variables (SVL at birth, recapture day and date of birth) were mean-centred. Where interactions were non-significant we present results from reduced models, excluding the interaction term.

All analyses were conducted in R (R Core Team, 2017). GLM and GLMM models were performed using the "lme4" package (Bates et al., 2014) with P-values, F-statistics and approximate denominator degrees of freedom derived based on Kenward-Roger's approximation, using the "lmerTest" package (Kuznetsova, Brockhoff, & Christensen, 2015) and type-II Wald X^2 tests and likelihood ratio tests using the "car" package (Fox *et al.*, 2017). Where significant differences between treatment groups were found, we tested for pairwise differences between them using

Tukey's HSD tests using the "multcomp" package (Hothorn, Bretz, Westfall, & Heidberger, 2008).

Results

Across the three study groups a total of 619 offspring were born from 166 litters (n = 107, 83 and 432 offspring from 33, 25 and 108 litters, from the 10-hour, 4-hour and field cohort groups, respectively). We found strong and significant differences in litter dates of birth among the three study groups ($F_{(2,174)}$ = 275.31, P < 0.0001). As predicted, there was a significant difference between the two laboratory treatments, with females in the 4-hour treatment giving birth significantly later than the 10-hour basking treatment (mean 14 February ± 1.03 SE days *vs.* mean 26 January ± 0.44 SE days; t = 11.41, P < 0.0001; see Fig. 1). Contrary to expectations, both laboratory treatments gave birth significantly earlier than the field cohort (mean 24 February ± 0.69 SE days; t = 23.36, P < 0.0001 and t = 7.03, P < 0.0001 for the 10-hour and 4-hour treatments, respectively).

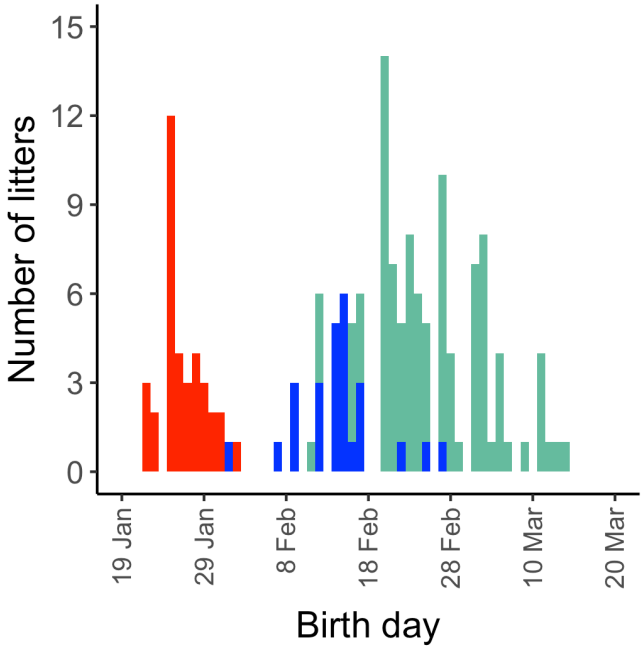

Figure 1: Histograms of birth date in a 10-hour (red) and a 4-hour (blue) laboratory basking availability treatments and a natural cohort (green; 2001/2) in a highland population of *Niveoscincus ocellatus*.

Dates of birth in the field cohort were later than is typical because the mean maximum temperature during the gestation period in the year of this study (2001/2) was substantially lower than in any other year of the long-term study (12.8 °C, see Fig. 2). Although dates of birth in the field cohort in this year were later than those experienced in any other year, they conformed to the long-term relationship between environmental temperature and birth date (Fig. 2). By comparing dates of birth in our experimental treatments to the long-term pattern (i.e., the thermal reaction norm), we estimated environmental temperature equivalents of the two laboratory basking treatments as 13.95 °C and 16.94 °C for the 4-hour and 10-hour treatments, respectively. Thus, basking treatments corresponded to environmental temperatures at either end of the range of those usually experienced by this population (approximately 1.5 °C from the long-term mean; see Fig. 2), but warmer than the temperatures experienced the field cohort in the year of the study.

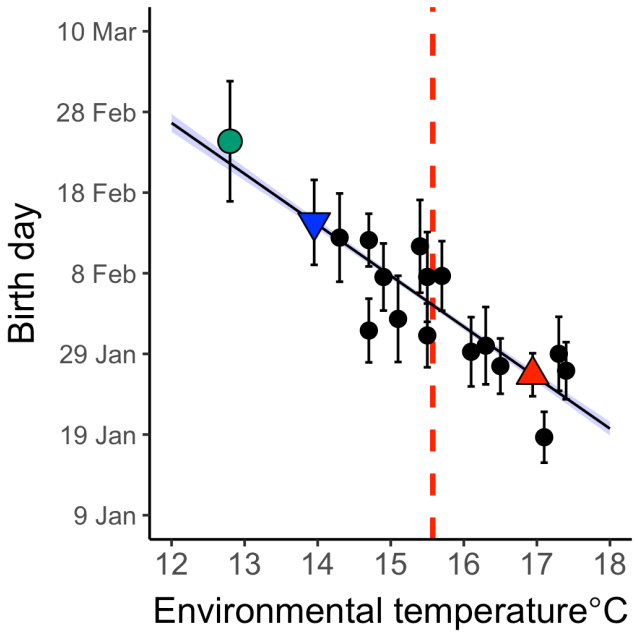

Figure 2: Relationship between mean maximum daytime temperature during the gestation period and birth date in a highland population of *Niveoscincus ocellatus* across a 16-year long-term study. The field cohort of the study year (2001/2) is shown as a green circle, remaining years as black circles. Temperature equivalents, based on birth dates, of two laboratory basking treatments are shown as a blue downwards-facing triangle (4-hour) and a red upwards-facing triangle (10-hour). Red dashed line indicates the long-term mean temperature (1985 – 2016) at this site. Error bars are standard deviations from the mean.

Size of offspring at birth differed significantly among study groups ($F_{(2,180.4)}$ = 14.161, P < 0.001, Fig. 3A); offspring from the field cohort where somewhat smaller (mean 29.55 ± 0.04 SE mm) than in either laboratory treatment (z = -4.56, P < 0.001 and z = -3.74, P < 0.001 against the 10-hour (mean 30.21 ± 0.08 SE mm) and 4-hour (mean 30.31 ± 0.10 SE mm) treatments, respectively). Offspring size did not significantly differ between basking treatments (z = -0.33, P = 0.94). Despite the substantial differences in birth date between the three study groups, we found no significant difference between the study groups in the probability of litters containing non-viable offspring (X^2 = 1.237, df = 2, P = 0.539). The 10-hour and 4-hour treatment groups and the field cohort included 6/38 (16%), 5/29 (17%) and 13/121 (11%) litters with non-viable foetuses or neonates respectively. Similarly, we did not find differences in offspring whole-body performance (measured as maximum sprint speed) between the three study groups ($F_{(2,101.0)}$ = 1.49, P = 0.231, Fig. 3B), nor was performance significantly affected by offspring size ($F_{(1,158.3)}$ = 0.37, P = 0.231).

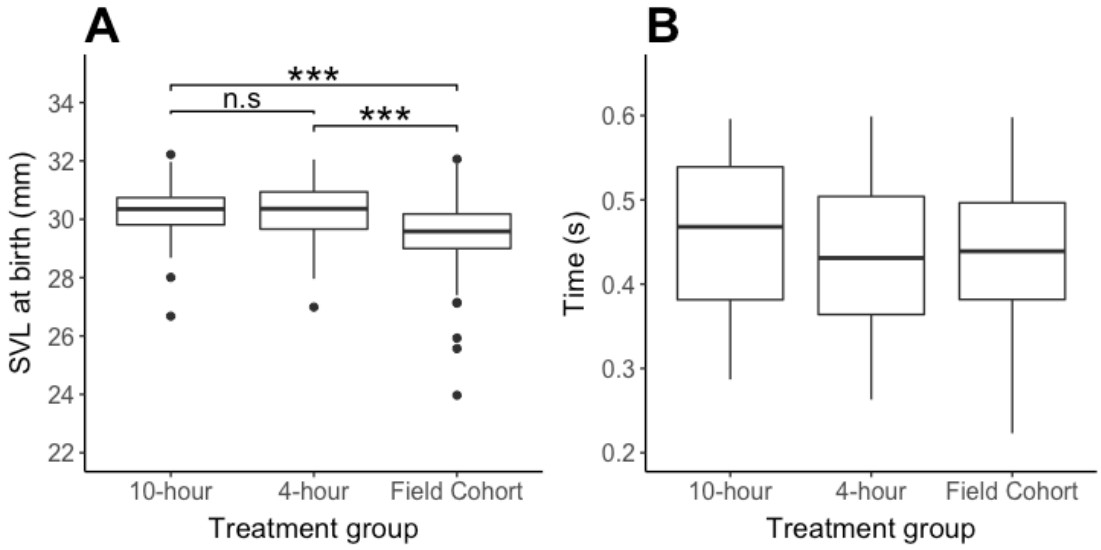

Figure 3: Differences at birth in A) SVL and B) sprint speed over 25 cm between two laboratory basking treatments and a field cohort (2001/2) of *Niveoscincus ocellatus*. Stars indicate highly significant differences between study groups.

Of the 619 total offspring released, 38/107 (36.5%), 24/83 (28.9%) and 112/432 (25.9%) were recaptured from the 10-hour, 4-hour and field cohort groups, respectively. While these results may suggest an increase in survivorship with temperature experienced during gestation, this failed to reach statistical significance (Table 1). Indeed, we found no effect of any of our predictors (date of birth, study group, or their interaction) on the probability of offspring being recaptured following winter (Table 1). We also did not find an effect of study group, or an interaction between study group and date of birth on SVL at recapture. However, we did find that date of birth significantly affected offspring size at recapture (Table 1).

Table 1: Summary of statistical results from GLMM (probability of recapture) and LMM (SVL at recapture) analyses of the effect of offspring characteristics and treatment across two laboratory basking treatments and a field cohort (2001/2) of a highland population of *Niveoscincus ocellatus.* Effect sizes are shown as means ± standard errors, significant effects are shown in bold.

	Recapture date	SVL at birth	Date of birth	Treatment group			Treatment – Date of birth interaction
				4-hour	10-hour	Field cohort	
Probability of recapture	-	0.089 ± 0.104 X^2 = 0.735 P = 0.391	0.001 ± 0.015 X^2 = 0.004 P = 0.952	-0.946 ± 0.427	-0.572 ± 0.384	-1.038 ± 0.472	-
				X^2 = 0.100; P = 0.606			X^2 = 0.930 P = 0.628
SVL at recapture	**0.136 ± 0.011** **$F_{(1,168)}$ = 149.73** **P < 0.0001**	**1.001 ± 0.117** **$F_{(1,168)}$ = 72.72** **P < 0.0001**	**-0.096 ± 0.016** **$F_{(1,168)}$ = 38.17** **P < 0.0001**	35.887 ± 0.437	35.877 ± 0.377	35.901 ± 0.492	-
				$F_{(2,168)}$ = 0.001; P = 0.999			$F_{(1,166)}$ = 0.31 P = 0.732

Early-born offspring were larger than late-born offspring with each additional day birth was delayed reducing offspring size at recapture by 0.10 ± 0.02 SE mm (Table 1, Fig. 4A). Our two covariates, SVL at birth and date of recapture, also had a significant effect on offspring size at recapture. For each increase of 1 mm in SVL at birth, SVL at recapture was 1.00 ± 0.12 SE mm greater (Table 1, Fig. 4B). Offspring recaptured later were larger than those recaptured earlier; each additional day before recapture increased size at recapture by 0.14 ± 0.01 SE mm (Table 1). We found that study group did not affect offspring size at recapture either independently, or in interaction, with birth date (Table 1).

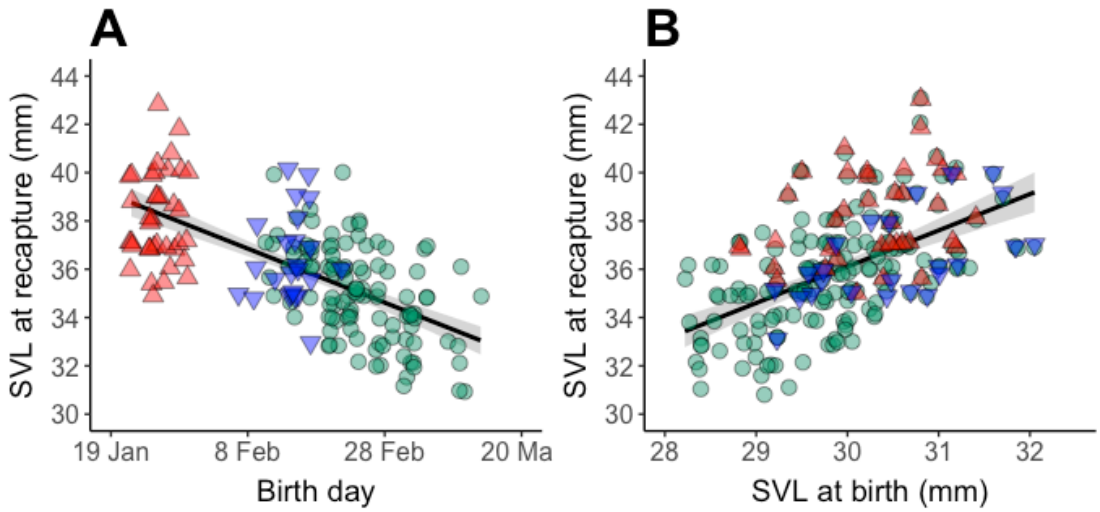

Figure 4: Relationship between offspring SVL following winter and A) birth date and B) SVL at birth in a field cohort (green circles) and two laboratory basking treatments (4-hour: blue downwards-facing triangle and 10-hour: red upwards-facing triangle) of a highland population of *Niveoscincus ocellatus*. Note: points have been jittered to minimise over-plotting.

Discussion

At a fundamental level, temperatures at the extreme ends of a species' reaction norm should have implications for normal developmental processes. Although thermal reaction norms can be broad, and can vary between populations due to local adaptation, development is usually optimised within a thermal range, beyond which organisms exhibit higher incidences of mortality and/or malformations, and reduced viability (Hare, Daugherty, & Cree, 2002; Ji, Qiu, & Dong, 2002; Lin, Ji, Luo, & Ma, 2005). In this study we found limited evidence that exposure to thermal conditions at the margins of a cold-adapted population's characterised thermal reaction norm impacted normal developmental processes. We did not find any evidence that exposure to these conditions influenced either the proportion of non-viable offspring or the whole body performance of offspring. The lack of an effect of thermal treatment on locomotor performance we found contrasts with temperature effects found in a range of species across reptile taxa, in which both increases and decreases in performance have been reported (see Shine & Olsson, 2003; Booth, 2006; While, Uller, & Wapstra, 2009). Combined, these results suggest that differences in basking availability during development among our treatment groups was not sufficient to push these lizards beyond their thermal range and thus compromise normal

development (see also Booth, 2006, but see Cadby *et al.*, 2014). Indeed, although exposure to unusually warm and cold conditions affected both birth date and offspring size, these effects were not outside the normal phenotypic range (e.g., Wapstra & Swain, 2001; Uller *et al.*, 2011; Cadby *et al.*, 2014).

There are a number of potential reasons extreme thermal conditions did not disrupt development. Individuals within this population may be adapted to temperature fluctuations beyond our characterised thermal reaction norm. Cold alpine sites (including our site; Pen *et al.*, 2010) often exhibit substantial fluctuations in temperatures both within and between years. Indeed, although the conditions experienced in this year fell outside those we have observed in any other year of the long-term study, they are likely to have been relatively frequent over longer historical time scales. Furthermore, while this population sits at the cooler end of the species' thermal distribution, we have previously shown that it is part of a larger sub-clade that includes lowland populations, from which it has diverged comparatively recently (Cliff *et al.*, 2015). During the last ice age, it is likely that the climate at our highland site was not suitable for *N. ocellatus*. Thus, this population may not have had sufficient time to evolve a strongly locally-adapted reaction norm (but see Cadby *et al.*, 2014 for evidence of local adaptation in other traits). Second, *Niveoscincus ocellatus* is viviparous and, therefore, has the ability to mediate the thermal conditions embryos are exposed to via behavioural thermoregulation. Females may, therefore, be able to buffer developing offspring against the effects of extreme temperatures that would otherwise lead to developmental stress. Indeed, previous work on this and other *Niveoscincus* species has shown that high altitude populations and species show plastic thermoregulatory responses to the prevailing thermal conditions that help maintain optimal developmental temperatures during gestation (e.g., Uller *et al.*, 2011; Cadby *et al.*, 2014; Caldwell, While, & Wapstra, 2017). Combined this suggests that individuals within this population may have several mechanisms by which normal development can be maintained, even under extreme conditions.

Independent of the effects on normal development, our exposure of females to developmental temperatures at the extremes of the species thermal reaction norms, nevertheless, had important effects on offspring phenotypes. Specifically, we found strong effects on the thermal conditions experienced during gestation on both

developmental rate (and consequently birth date) and on offspring size. These effects are consistent with previous work on this species (see Wapstra, 2000; Cadby *et al.*, 2014; Chapter 2: Cunningham *et al.*, 2017), and on reptiles more generally (Caley & Schwarzkopf, 2004; see Booth, 2006). Importantly, these effects, particularly on dates of birth, had significant consequences for fitness related traits. We found that early-born offspring were larger at recapture than late-born offspring. Date of birth and growth rates may have significant long-term consequences later in life. For instance, we have previously shown that earlier birth dates can result in larger size at maturation in *N. ocellatus* (Pen *et al.*, 2010). This pattern of early birth date conferring an advantage later in life is observed across vertebrates, with evidence in some species of mammals and many bird species (e.g., Green & Rothstein, 1993; Arnold, Hatch, & Nisbet, 2004; Saino *et al.*, 2012; Plard *et al.*, 2015). While growth rates were affected by birth date, we found no effect on offspring survival over the first winter. This is consistent with previous work that suggests limited selection on offspring birth date (Uller *et al.*, 2011). However, in contrast to previous work, we failed to find positive selection on offspring body size and date of birth (see Atkins *et al.*, 2007; Uller *et al.*, 2011). This could either be because the phenotypic effects of gestation temperatures in the current study did not push phenotypes far enough to have significant consequences on survival itself. Alternatively, climatic conditions during the period of this study may not have been severe enough to impose significant selection on offspring size at birth or date of birth. More work is required to examine the context dependent ways in which the phenotypic effects of thermal conditions during development can mediate fitness in both the short- and the long-term (While *et al.*, in press).

There are a number of mechanisms, which may explain the positive effect of birth date on early growth. First, an increased activity period prior to winter resulting from early birth, likely provides the opportunity for increased growth before winter (Wapstra *et al.*, 2001; Wapstra *et al.*, 2010). Alternatively, these effects may be more indirect. For example, if early birth dates correlate with increased resource availability. Indeed, if phenologies of the biotic components of the ecosystem are affected to different extents by changes in temperature (e.g., food availability or predation pressure), this can affect growth and/or survival (i.e., trophic mismatches; Harrington, 1999). Thus, reduced growth in the field cohort may be explained if

phenologies of other components of the ecosystem were not delayed to the same extent. However, because *N. ocellatus* is a trophic generalist and can, therefore, opportunistically take advantage of a diverse range of food sources which are available at different periods (Wapstra & Swain, 1996) it is unlikely to suffer a catastrophic mismatch between birthdate and food availability (Bohm *et al.*, 2016), at least within the range of thermal conditions we explored. Further experimental work is required to tease out the relative contributions of the mechanisms underpinning these effects.

The results from this study have important implications for our ability to predict the consequences of global climate change. Warming climates are often predicted to have negative consequences for a range of species, especially populations of ectotherms at the extremes of a species' climatic distribution. However, our study shows that increasing temperatures may have positive consequences for our population, because resultant advances in dates of birth result in increased offspring size. These effects are in line with a growing body of literature that suggests that global warming may, at least initially, have a number of positive effects on organismal fitness in ectotherms and, thus, on population persistence (Chamaillé-Jammes, Massot, Aragon, & Clobert, 2006; Wapstra *et al.*, 2009; While & Uller, 2014). Specifically, species that exhibit characteristics such as a generalist diet and/or adaptations to variable conditions are likely to be at comparatively low risk (see Bohm *et al.*, 2016). This study emphasises the importance of characterising and understanding thermal reaction norms of species for predicting how changes in climate may affect population persistence. To fully understand these effects, we need more studies investigating the multi-faceted ways in which climate influences organismal fitness. Only then can we accurately project how populations, species and communities will persist under ongoing climate change.

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Ethics

All guidelines and procedures for the use of animals were approved by the University of Tasmania Animal Ethics Committee (A0017006, A0016736).

Literature Cited

Arnold, J. M., Hatch, J. J., & Nisbet, I. C. (2004). Seasonal declines in reproductive success of the common tern Sterna hirundo: timing or parental quality?. *Journal of Avian Biology*, **35**, 33-45. doi: 10.1111/j.0908-8857.2004.03059.x

Atkins, N., Swain, R., Wapstra, E., & Jones S. M. (2007). Late stage deferral of parturition in the viviparous lizard *Niveoscincus ocellatus* (Gray 1845): implications for offspring quality and survival. *Biological Journal of the Linnean Society*, **90**, 735-746. doi: 10.1111/j.1095-8312.2007.00770.x

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H., & Grothendieck, G. (2014). Package 'lme4'. R foundation for statistical computing, Vienna, 12.

Böhm, M., Cook, D., Ma, H., Davidson, A. D., Garcia, A., Tapley, B., Pearce-Kelly, P., & Carr, J. (2016). Hot and bothered: Using trait-based approaches to assess climate change vulnerability in reptiles. *Biological Conservation*, **204**, 32-41. doi: 10.1016/j.biocon.2016.06.002

Booth, D. T. (2006) Influence of incubation temperature on hatchling phenotype in reptiles. *Physiological and Biochemical Zoology*. **79**, 274-281. doi: 10.1086/499988

Cadby, C. D., Jones, S. M., & Wapstra, E. (2014). Geographical differences in maternal basking behaviour and offspring growth rate in a climatically widespread viviparous reptile. *Journal of Experimental Biology*, **217**, 1175-1179. doi: 10.1242/jeb.089953

Caldwell, A. J., While, G. M., & Wapstra, E. (2017). Plasticity of thermoregulatory behaviour in response to the thermal environment by widespread and alpine reptile species. *Animal Behaviour*, **132**, 217-227. doi: 10.1016/j.anbehav.2017.07.025

Caley, M. J., & Schwarzkopf, L. (2004). Complex growth rate evolution in a latitudinally widespread species. *Evolution*, **58**, 862-869. doi: 10.1554/03-493

Chamaillé-Jammes, S., Massot, M., Aragon, P., & Clobert, J. (2006). Global warming and positive fitness response in mountain populations of common lizards Lacerta vivipara. *Global Change Biology*, **12**, 392-402. doi: 10.1111/j.1365-2486.2005.01088.x

Chevin, L. M., Lande, R., & Mace, G. M. (2010). Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS biology*, **8**, e1000357. doi: 10.1371/journal.pbio.1000357

Cliff, H. B., Wapstra, E., & Burridge, C. P. (2015). Persistence and dispersal in a Southern Hemisphere glaciated landscape: the phylogeography of the spotted snow skink (*Niveoscincus ocellatus*) in Tasmania. *BMC evolutionary biology*, **15**, 121. doi: 10.1186/s12862-015-0397-y

Cohen, J. M, Lajeunesse, M. J., & Rohr, J. R. (2018) A global synthesis of animal phenological responses to climate change. *Nature Climate Change.* **8**, 1. doi: 10.1038/s41558-018-0067-3

Cunningham, G. D., While, G. M., & Wapstra, E. (2017). Climate and sex ratio variation in a viviparous lizard. *Biology Letters*, **13**, 20170218. doi: 10.1098/rsbl.2017.0218 (Chapter 2).

Fox, J., Weisberg, S., Adler, D., Bates, D., Baud-Bovy, G., Ellison, S., Firth, D., Friendly, M., Gorjanc, G., Graves, S., & Heiberger, R. (2017). Package 'car'.

Georges, A., Beggs, K., Young, J. E., & Doody, J. S. (2005). Modelling development of reptile embryos under fluctuating temperature regimes. *Physiological and Biochemical Zoology*, **78**, 18-30. doi: 10.1086/425200

Green, W. C., & Rothstein, A. (1993). Persistent influences of birth date on dominance, growth and reproductive success in bison. *Journal of Zoology*, **230**, 177-186. doi: 10.1111/j.1469-7998.1993.tb02680.x

Hare, K. M., Daugherty, C. H., & Cree, A. (2002). Incubation regime affects juvenile morphology and hatching success, but not sex, of the oviparous lizard *Oligosoma suteri* (Lacertilia: Scincidae). *New Zealand Journal of Zoology*, **29**, 221-229. doi: 10.1080/03014223.2002.9518306

Harrington, R., Woiwod, I., & Sparks, T. (1999) Climate change and trophic interactions. *Trends in Ecology and Evolution*, **14**, 146–150. doi: 10.1016/S0169-5347(99)01604-3

Hill., P., Ezaz, T., Wapstra, E., & Burridge, C. (2018). Conservation of sex-linked markers among conspecific populations of a viviparous skink, *Niveoscincus ocellatus*, exhibiting genetic and temperature dependent sex determination. *Genome Biology and Evolution.* ***in press***.

Hothorn, T., Bretz, F., Westfall, P., & Heiberger, R. (2008). Multcomp: simultaneous inference for general linear hypotheses.R Package Version 1.0-3.

Ji, X., Qiu, Q. B., & Diong, C. H. (2002). Influence of incubation temperature on hatching success, energy expenditure for embryonic development, and size and morphology of hatchlings in the oriental garden lizard, *Calotes versicolor* (Agamidae). *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, **292**, 649-659. doi: 10.1002/jez.10101

Jones, S. M., Wapstra, E., & Swain, R. (1997). Asynchronous male and female gonadal cycles and plasma steroid concentrations in a viviparous lizard, *Niveoscincus ocellatus* (Scincidae), from Tasmania. *General and Comparative Endocrinology*, **108**, 271-281. doi: 10.1006/gcen.1997.6971

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2015). Package 'lmerTest'. *R package version*, 2(0).

Lin, Z. H., Ji, X., Luo, L. G., & Ma, X. M. (2005). Incubation temperature affects hatching success, embryonic expenditure of energy and hatchling phenotypes of a prolonged egg-retaining snake, *Deinagkistrodon acutus* (Viperidae). *Journal of Thermal Biology*, **30**, 289-297. doi: 10.1016/j.jtherbio.2005.01.002

Löwenborg, K., Shine, R., & Hagman, M. (2011). Fitness disadvantages to disrupted embryogenesis impose selection against suboptimal nest-choice by female grass snakes, Natrix natrix (Colubridae). *Journal of Evolutionary Biology*, **24**, 177-183. doi: 10.1111/j.1420-9101.2010.02153.x

McCaffery, R. M., & Maxwell, B. A. (2010) Decreased winter severity increases viability of a montane frog. *Proceedings of the National Academy of Sciences*, **107**, 8644-8649. doi: 10.1073/pnas.0912945107

Merilä, J. (2012). Evolution in response to climate change: in pursuit of the missing evidence. *BioEssays*, **34**, 811-818. doi: 10.1002/bies.201200054

Moczeck, A. P., Sultan, S., Foster, S., Ledón-Rettig, C., Dworkin, I., Nijhout, H. F., & Pfennig, D. W. (2011). The role of developmental plasticity in evolutionary innovation. *Proceedings of the Royal Society B: Biological Sciences*, **278,** 2705-2713. doi: 10.1098/rspb.2011.0971

Monasterio, C., Shoo, L. P., Salvador, A., Iraeta, P., & Díaz, J. A. (2013). High temperature constrains reproductive success in a temperate lizard: implications for distribution range limits and the impacts of climate change. *Journal of Zoology*, **291**, 136-145. doi: 10.1111/jzo.12057

Noble, D. W., Stenhouse, V., & Schwanz, L. E. (2018). Developmental temperatures and phenotypic plasticity in reptiles: a systematic review and meta-analysis. *Biological Reviews*, **9**, 72-97. doi: 10.1111/brv.12333

Pearson, P. R., & Warner, D. A. (2016). Habitat-and season-specific temperatures affect phenotypic development of hatchling lizards. *Biology letters*, **12**, 20160646. doi: 10.1098/rsbl.2016.0646

Pen, I., Uller, T., Feldmeyer, B., Harts, A., While, G. M., & Wapstra, E. (2010). Climate-driven population divergence in sex-determining systems. *Nature*, **468**, 436. doi: 10.1038/nature09512

Phillimore, A. B., Stålhandske, S., Smithers, R. J., & Bernard, R. (2012). Dissecting the contributions of plasticity and local adaptation to the phenology of a butterfly and its host plants. *The American Naturalist*, **180**, 655-670. doi: 10.1086/667893

Plard, F., Gaillard, J. M., Coulson, T., Hewison, A. J., Douhard, M., Klein, F., Delorme, D., Warnant, C. & Bonenfant, C. (2015). The influence of birth date via body mass on individual fitness in a long-lived mammal. *Ecology*, **96**, 1516-1528. doi: 10.1890/14-0106.1

R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Saino, N., Romano, M., Ambrosini, R., Rubolini, D., Boncoraglio, G., Caprioli, M., & Romano, A. (2012). Longevity and lifetime reproductive success of barn swallow offspring are predicted by their hatching date and phenotypic quality. *Journal of Animal Ecology*, **81**, 1004-1012. doi: 10.1111/j.1365-2656.2012.01989.x

Scheiner, S. M. (1993). Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics*, **24**, 35-68. doi: 10.1146/annurev.es.24.110193.000343

Shine, R. & Olsson, M. (2003). When to be born? Prolonged pregnancy or incubation enhances locomotor performance in neonatal lizards (Scincidae). *Journal of Evolutionary Biology*, **16**, 823-832. doi: 10.1046/j.1420-9101.2003.00600.x

Shine, R., Elphick, M. J., & Harlow, P. S. (1997). The influence of natural incubation environments on the phenotypic traits of hatchling lizards. *Ecology*, **78**, 2559-2568. doi: 10.1098/rspb.1997.0065

Telemeco, R. S., Warner, D. A., Reida, M. K., & Janzen, F. J. (2013) Extreme developmental temperatures result in morphological abnormalities in painted turtles (*Chrysemys picta*): a climate change perspective. *Integrative Zoology*, **8**, 197-208. doi: 10.1111/1749-4877.12019

Uller, T. (2008) Developmental plasticity and the evolution of parental effects. *Trends in Ecology and Evolution*, **23**, 432-438. doi: 10.1016/j.tree.2008.04.005

Uller, T., While, G. W., Cadby, C. D., Harts, A., O'Connor, K., Pen, I., & Wapstra, E. (2011). Altitudinal divergence in thermoregulatory behaviour may be driven by differences in selection on offspring survival in a viviparous lizard. *Evolution*, **65**, 2313-2324. doi: 10.1111/j.1558-5646.2011.01303.x

Visser, M. E. (2008). Keeping up with a warming world; assessing the rate of adaptation to climate change. *Proceedings of the Royal Society of London B: Biological Sciences*, **275**, 649-659. doi: 10.1098/rspb.2007.0997

Visser, M. E. & Both, C. (2005). Shifts in phenology due to global climate change: the need for a yardstick. *Proceedings of the Royal Society of London B: Biological Sciences*, **272**, 2561-2569. doi: 10.1098/rspb.2005.3356

Wapstra, E. (2000). Maternal basking opportunity affects juvenile phenotype in a viviparous lizard. *Functional Ecology*, **14**, 345-352. doi: 10.1046/j.1365-2435.2000.00428.x

Wapstra, E. & Swain, R. (2001). Geographic and annual variation in life-history traits in a temperate zone Australian skink. *Journal of Herpetology*, **35,** 194-203. doi: 10.2307/1566108

Wapstra, E. & Swain, R. (1996). Feeding ecology of the Tasmanian spotted skink, *Niveoscincus ocellatus* (Squamata: Scincidae). *Australian Journal of Zoology*, **44**, 205-213. doi: 10.1071/ZO9960205

Wapstra, E., Swain, R., & O'Reilly J. M. (2001). Geographic variation in age and size at maturity in a small Australian viviparous skink, *Copeia*, **3**, 646-655. doi: 10.1643/0045-8511(2001)001[0646:GVIAAS]2.0.CO;2

Wapstra, E., Swain, R., Jones, S. M., & O'Reilly, J. (1999). Geographic and annual variation in reproductive cycles in the Tasmanian spotted snow skink, *Niveoscincus ocellatus* (Squamata: Scincidae). *Australian Journal of Zoology*, **47**, 539-550. doi: 10.1071/ZO99038

Wapstra, E., Uller, T. While, G. W., Olsson, M., & Shine, R. (2010) Giving offspring a head start in life: field and experimental evidence for selection on maternal basking behaviour in lizards. *Journal of Evolutionary Biology*, **23**, 651-657. doi: 10.1111/j.1420-9101.2009.01924.x

Wapstra, E., Uller, T., Sinn, D. L., Olsson, M., Mazurek, K., Joss, J., & Shine, R. (2009). Climate effects on offspring sex ratio in a viviparous lizard. *Journal of Animal Ecology*, **78**, 84-90. doi: 10.1111/j.1365-2656.2008.01470.x

Warner, D. A. & Shine, R. (2008). Maternal nest-site choice in a lizard with temperature-dependent sex determination. *Animal Behaviour*, **75**, 861-870. doi: 10.1038/nature06519

Willette, D. A., Tucker, J. K., & Janzen, F. J. (2005). Linking climate and physiology at the population level for a key life-history stage of turtles. *Canadian Journal of Zoology*, **83**, 845-850. doi: 10.1139/z05-078

While, G. M. & Uller, T. (2014). Quo vadis amphibia? Global warming and breeding phenology in frogs, toads and salamanders. *Ecography*, **37**, 921-929. doi: 10.1111/ecog.00521

While, G. M., Uller, T., & Wapstra, E. (2009). Offspring performance and the adaptive benefits of prolonged pregnancy: experimental tests in a viviparous lizard. *Functional Ecology*, **23**, 818-825. doi: 10.1111/j.1365-2435.2009.01544.x

While, G. M., Noble, D. W. A., Uller, T., Warner, D. A., Riley, J. E., Du, W. G., & Schwanz, L. E. (2018) Patterns of developmental plasticity in response to the thermal incubation environment in reptiles. *Journal of Experimental Zoology*. ***in press***.

While, G.M., Williamson, J., Prescott, G., Horváthová, T., Fresnillo, B., Beeton, N.J., Halliwell, B., Michaelides, S., & Uller, T. (2015). Adaptive responses to cool climate promotes persistence of a non-native lizard. *Proceedings of the Royal Society of London B: Biological Sciences*, **282**, 20142638. doi: 10.1098/rspb.2014.2638

Yntema, C. L. (1960) Effects of various temperatures on the embryonic development of *Chelydra serpentina*. *Anatomical Record*, **136**, 305-306.

Chapter 4, Appendix:

Here, I present the results of linear mixed models (LMMs) with significance of main effects fit by maximum likelihood (ML) rather than REML, as in the text of chapter 4. When LMMs were fit in this way significance of all tested effects remained the same as presented in the text and effect sizes were substantially the same. Thus, the conclusions reached in this chapter are not affected by this alternative analysis.

Size of offspring at birth differed significantly among study groups ($F_{(2,183.16)} = 14.41$, $P < 0.001$, $\sigma_{ID} = 0.54 \pm 0.74$ SD, $\sigma_{resid} = 0.34 \pm 0.58$ SD); offspring from the field cohort where smaller (mean 29.55 ± 0.04 SE mm) than in either laboratory treatment ($z = -4.59$, $P < 0.001$ and $z = -3.78$, $P < 0.001$ against the 10-hour (mean 30.21 ± 0.08 SE mm) and 4-hour (mean 30.31 ± 0.10 SE mm) treatments, respectively). Offspring size did not significantly differ between basking treatments ($z = -0.32$, $P = 0.95$, $\sigma^2_{ID} = 0.00 \pm 0.02$ SD, $\sigma^2_{resid} = 0.01 \pm 0.08$ SD). There was no significant difference in offspring whole-body performance (maximum sprint speed; $\sigma_{ID} = 0.00 \pm 0.02$ SD, $\sigma_{resid} = 0.00 \pm 0.01$ SD) among the three study groups ($F_{(2,104.0)} = 1.56$, $P = 0.216$) nor was performance significantly affected by offspring size ($F_{(1,159.5)} = 0.33$, $P = 0.566$).

Table A1: Summary of statistical results LMM (SVL at recapture) analyses of the effect of offspring characteristics and treatment across two laboratory basking treatments and a field cohort (2001/2) of a highland population of *Niveoscincus ocellatus*. Effect sizes are shown as means ± standard errors, significant effects are shown in bold. Variance components for the GLMM test of the effect of offspring characteristics on the probability of recapture from the models presented in the main text are shown below, since these models were not re-analysed.

	Recapture date	SVL at birth	Date of birth	Treatment group			Treatment – Date of birth interaction
				4-hour	10-hour	Field cohort	
SVL at recapture	**0.136 ± 0.011** **$F_{(1,174)} = 155.08$** **$P < 0.0001$**	**1.001 ± 0.115** **$F_{(1,174)} = 75.31$** **$P < 0.0001$**	**-0.096 ± 0.015** **$F_{(1,174)} = 39.53$** **$P < 0.0001$**	35.887 ± 0.437	35.877 ± 0.377	-1.038 ± 0.472	-
				$F_{(2,168)} = 0.001$; $P = 0.999$			$F_{(2,174)} = 0.33$ $P = 0.721$
			$\sigma_{ID} = 0.00 \pm 0.00$ SD $\sigma_{resid} = 1.37 \pm 1.17$ SD				$\sigma_{ID} = 0.00 \pm 0.00$ SD $\sigma_{eresid} = 1.36 \pm 1.17$ SD
Probability of recapture			$\sigma_{ID} = 0.00 \pm 0.00$ SD				$\sigma_{ID} = 0.00 \pm 0.00$ SD

Chapter 5:

Degrees of Change: Between and Within Population Variation in Thermal Reaction Norms of Phenology in a Viviparous Lizard.

Manuscript status: Cunningham, G.D., While, G.M., Olsson, M., Ljungström, G. & Wapstra, E. ***in prep.***

Abstract

As climates warm, species and the populations that make them up will be faced with novel climatic environments to which they may not be adapted. Populations can be buffered against the negative effects or maximise the beneficial effects of altered conditions, in the short-term via phenotypic plasticity and/or in the longer term, through adaptive evolution. However, due to local adaptation and biogeographic and demographic history, populations are likely to differ in mean-level responses across environmental gradients, and the degree to which responses vary between individuals. To accurately predict the consequences of climatic change for species and population persistence, we require estimates of population-specific patterns of among-individual variation in plastic responses. Here we used a long-term dataset tracking births over 16 years in two climatically distinct populations of the viviparous skink *Niveoscincus ocellatus* to investigate inter- and intra- population variability in the response of parturition date to environmental temperature under an animal model framework. We found that, whilst the two populations share a common population-mean reaction norm, they differ substantially in the among-individual variability of reaction norms at the phenotypic level. These results suggest that, even where populations share a common mean-level response, we cannot assume that that they will be affected similarly by altered conditions. If we are to accurately assess how changing climates will impact species and populations, we require estimates of how plastic responses vary both within and among populations.

Introduction

Climate change is affecting the ecological and climatic context within which species exist. A common response of species to changing climates is a shift in the timing of life history events (i.e., phenology). Such changes, including the timing of flowing (e.g., Fitter & Fitter, 2002), migration (e.g., Hüppop & Hüppop, 2003; Sparks *et al.*, 2005), laying and spawning (e.g., Beebee, 1995; Crick *et al.*, 1997), birth and hatching (e.g., Visser & Holleman, 2001; Husby *et al.*, 2010), and the length of growing seasons (e.g., Menzel & Fabian, 1999), have been reported for a range of species (e.g., Parmesan & Yohe, 2003; Root *et al.*, 2003; Menzel *et al.*, 2006; Parmesan, 2007; Charmantier *et al.*, 2008; Lane *et al.*, 2012; While & Uller, 2014; Ge *et al.*, 2016; Cohen *et al.*, 2018). Importantly, these effects can have either positive or negative consequences for population persistence (Charmantier *et al.*, 2008; Lane *et al.*, 2012). For example, differences in the magnitude of phenological shifts between animals and their food sources can lead to mismatches between periods when resource requirements are high (e.g., during periods of parental provisioning) and the availability of these resources (e.g., Visser & Both, 2005; Willette, *et al.*, 2005; Lane *et al.*, 2012; Thackeray *et al*., 2016; Noble *et al.*, 2018). In extreme cases, such phenological mismatches can lead to population collapse (Visser & Both, 2005; Willette, Tucker, & Janzen, 2005; Noble *et al.*, 2018). Conversely, changes in phenology can have positive effects. For example, earlier parturition dates or increased length of activity seasons may enhance population growth because more time is available to juveniles for condition building and growth (McCaffery & Maxwell, 2010; Phillimore *et al.*, 2012). Understanding how species' phenologies are affected by climate, and the consequences of these for individual fitness is, therefore, critical to assess population and species susceptibility to changing climatic conditions.

Because altered phenologies can have significant consequences for population viability, an increasing number of assessments of the responses of species to climate change take shifts in phenology into account (e.g., Mitchell *et al.*, 2008; Chapman *et al.*, 2017; Vicenzi, 2017; Dupuite, 2015). However, in so doing, they often assume (explicitly or implicitly) that responses will be homogeneous among populations, and among the individuals within them. Such assessments also often ignore the capacity of these responses to evolve due to novel selective pressures, assuming that the

relationship between environmental temperature and phenology (i.e., the thermal reaction norm of parturition) will be stable, both geographically and temporally (Moran *et al.*, 2015). There are several reasons, however, that we should be sceptical that these assumptions hold, especially where populations are distributed across a broad climatic or geographical range (Valladares *et al.*, 2014, Noble *et al*., 2018). Variation in responses among and within populations should be expected due to local adaptation, varying degrees of connectivity, founder effects, past population bottlenecks, and/or genetic drift (Kawecki, 2004; Kawecki & Ebert, 2008; Mägi *et al.*, 2011).

Differences in plastic responses between populations can affect predictions of how species will be impacted by changing environments (Bolnick, *et al.*, 2011; Valladares *et al.*, 2014; Moran *et al.*, 2016). It is, therefore, important that we understand how phenological reaction norms vary both within and between populations if we are to accurately gauge the susceptibility of populations and species to the effects of climate change. Whilst some studies have investigated how phenological reaction norms vary between populations (e.g., Porlier *et al.*, 2012; Rutschmann *et al.*, 2016) and within populations (e.g., McGaugh *et al.*, 2010; Ljungström *et al.*, 2015), few have been able to compare between-individual variability among populations (but see Husby *et al.*, 2010; Edge *et al.*, 2017). This lack of information is, perhaps, unsurprising, because such studies require long-term longitudinal datasets that include repeated measures of individuals across a range of conditions and across multiple populations. Data of this type exist, however, for few species.

Here, we used a long-term dataset, tracking births in populations located at the extreme climatic limits (i.e., in warm lowland and cold alpine areas) of the spotted snow skink *Niveoscincus ocellatus* across 16 seasons, to investigate how thermal reaction norms of parturition date vary within and between populations. We have previously shown that these populations share a similar population-level reaction norm (Chapter 2: Cunningham *et. al,* 2017). This similarity of response is surprising, given that they experience very different environmental conditions, and that they differ substantially in a number of traits including size and age at maturity (Wapstra *et al.*, 2001), size at birth (Uller *et al.*, 2011), preferred body temperature (Cadby *et al.*,

2014), sex determination system (Chapter 2: Cunningham *et al.*, 2017) and basking behaviour (Uller *et al.*, 2011; Caldwell *et al.*, 2017). Such differences in thermoregulatory behaviour during gestation are important to consider in *N. ocellatus* because this species is viviparous. Therefore, rates of development, and consequently parturition dates, result from an interaction between physiology and basking behaviour throughout gestation. Whilst these populations share a common mean-level plastic response of parturition date to temperature, we do not know the extent to which these plastic responses vary among individuals within populations.

To address the question of how the plastic response of individuals varies within populations, and to estimate how mean-level parturition dates will be affected by increasing temperatures, we first used a 16-year, long-term dataset to characterise population-mean level reaction norms. We then estimated the among-individual variation in individual thermal reaction norms of parturition date within populations using an 'animal model' approach (Henderson, 1950; Kruuk, 2004). By expanding this approach into a bivariate framework we then tested whether among-individual variation in individual reaction norms differed between populations. By incorporating pedigree information we also estimated the degree to which reaction norms were heritable within populations. We note, however, that because our pedigree information was limited, the heritability estimates we present should be treated as preliminary estimates only.

Shifts in phenology can have significant effects on fitness (e.g., Charmantier *et al.*, 2008; Lane *et al.*, 2012). Because the strength of selection on parturition dates likely differs between sites, and because the sites differ in a number of environmental characteristics, we predicted that among-individual variation in reaction norms, at both the phenotypic and genetic levels, would be different in these populations. Specifically, we predicted that phenotypic and genetic variation would be lower in the extreme cold highlands than the warm, relatively benign lowlands because lowland sites are more environmentally heterogeneous than the highlands and because stronger selection on development in the highlands would result in increased canalisation of thermal reaction norms in this population (see Uller *et al.*, 2011).

Materials and Methods

Study system and population monitoring

Niveoscincus ocellatus (Gray, 1845) is a small viviparous skink, (3-10g, 60-80mm snout-vent length) endemic to Tasmania, Australia. Annually, between 2000/1 and 2015/16, during the austral summer, we monitored births in two populations located at the extreme altitudinal and thermal limits of the species' range, according to a standard field and laboratory protocol (Wapstra *et al.*, 2009; Uller *et al.*, 2011; Cadby *et al.*, 2014). The highland site is located at Lake Augusta, on Tasmania's Central Plateau (41°86'S, 146°53'E, elevation: 1150 m) whilst the lowland population is located near to Orford, on the east coast (42°55'S, 147°87'E, elevation: 30 m). Both sites are surrounded by either unsuitable habitat or road and there is, consequently, little immigration or emigration in either population (Wapstra *et al.*, 2010; Uller *et al.*, 2011; Cadby *et al*., 2010). Each year the majority (90 – 95%) of pregnant females were captured late in the gestation period but prior to birth (mid-December and mid-January for the lowlands and highlands respectively), in order to minimise time spent in the laboratory. Following capture, females were identified using toe-clips and their capture location was recorded (± 5 m). Females were then returned to a temperature-controlled facility (ambient temperature 16 °C) at the University of Tasmania, where they were weighed (± 1 mg) and housed in individual terraria (600 cm^2) with shelter, a basking rock and a basking light provided. Water was available *ad libitum* and lizards were fed 3-4 times per week on mealworms (*Tenebrio* larvae) and Heinz baby food, supplemented with protein powder (Nature's Way). Terraria were checked twice daily for newborns and, following birth, mothers and juveniles were weighed and measured and offspring were sexed and toe-clipped for future identification. Within one week of birth, mothers and offspring were released at one of 12 locations within each site. Mothers were released at the closest release location to where they were captured, whilst juveniles were randomly allocated to one of these release locations. Thus, we avoided conflating the effects of subclimates within sites with heritability because, following birth, offspring did not occupy the same location within sites as their mothers or siblings. This dataset now covers 16 years of

uninterrupted data, consisting of records for over 1200 litters, from over 600 unique females, from each population. Many of these females were born during the study period and, thus, have known maternity and siblings and many have been repeatedly captured across multiple years (see Table 1).

Table 1: Summary information of parturition data across 16 years (2000/2001 – 2015/2016) in a lowland and a highland population of the viviparous skink *Niveoscincus ocellatus*.

Population	Number of litters	Unique mothers			Number of recorded litters per unique mother				Number of daughters subsequently recorded as mothers per unique mother			
		Total	Known parturition date	Mother's parturition date known	1	2	3	>3	1	2	3	>3
Lowland	1463	694	190	40	369 (94)	137 (41)	73 (21)	115 (34)	108	29	8	0
Highland	1230	642	252	86	351 (128)	155 (69)	62 (26)	64 (29)	132	36	12	1

Note: Figures in parentheses indicate the number of litters recorded per unique mother with known parturition date.

Population-level patterns

To explore population mean-level response of parturition dates to temperature we first fit a linear mixed model (LMM) with cohort-mean parturition date as a response variable, with the site-specific mean maximum daytime temperature during the gestation period (October 1 - December 30 and October 15 - January 15 for the lowlands and highlands, respectively), site (highland or lowland), and their interaction as fixed factors, and with year as random factor. Temperature data were obtained from Australian Bureau of Meteorology stations located close to each of the sites. (Australian Bureau of Meteorology website).

Within-population patterns

To explore patterns of phenotypic variation in the effect of temperature on parturition date among individuals within populations we used an "animal model" framework (Henderson, 1950; Kruuk, 2004). Using univariate random regression models we estimated amongst-individual variation in mean parturition dates and their response to temperature during gestation for each population by fitting constant (x = 0) and linear (x = 1) polynomial functions (Φ). A first order function estimates a linear reaction

norm model for individual parturition dates across temperature. Thus among-individual variance in intercepts (V_{I}) and slopes (V_{IxE}) of reaction norms are estimated, as well as the covariance between slopes and intercepts, based on repeated observations of females across years, resulting in a 2x2 variance-covariance matrix. In our analysis we used Legendre polynomials, which are only defined within the range -1 to +1. We, therefore, standardised site-specific measurements of temperature (see above) to be within this range using the equation:

$$T = -1 + 2\left(T_{\mathrm{gest}} - minT_{\mathrm{gest}}\right)/\left(maxT_{\mathrm{gest}} - minT_{\mathrm{gest}}\right)$$

Our full model was:

$$PD_{i,} = XT_i + Z_1\varphi(I_i, n_1, T) + Z_2Yr_i + e_i$$

In this model, PD_i is the vector of individual parturition dates and X is the design matrix relating to the fixed effect of standardised yearly mean maximum daytime temperature during gestation (T on the range -1 to 1, see above). Z_1 and Z_2 are the design matrices relating to the random effects of individual female (I_i), and year (Yr_i) observations, respectively. Year (Yr_i vector) was included as a random effect to model variation among years not explained by environmental temperature. Thus, $\varphi(I_i, n_1, T)$ is the random regression function of order n_1 of individual i. We modelled both homogeneous and heterogeneous by-year residual error variances. In all cases a heterogeneous structure gave a better fit than a homogeneous structure (Lowland: $\chi^2_{15} = 90.18$, $P < 0.0001$; Highland: $\chi^2_{15} = 90.30$, $P < 0.0001$) comparing model 4 (Table 2) fit with heterogeneous and homogenous residual errors for the lowland and highland sites, respectively. We, therefore, present results from models fit with heterogeneous error variances. To test the significance of model fit and random factors, we fit models of increasing variance structure complexity (see Table 2) and tested the significance of variance and covariance components using likelihood ratio tests (LRTs) with degrees of freedom equal to the difference in degrees of freedom between the models being compared.

Between-population comparison

To test whether among-individual variation in thermal reaction norms of parturition date differed between populations, we modelled the response of parturition date to temperature during gestation in a bivariate random regression model. To do this we combined the datasets from both populations, treated parturition date in each population as a separate trait and included population-specific scaled standardised environmental temperature (see above) as a fixed effect. In these models we constrained the covariance between population-specific traits to be zero since, given the distance between sites and the limited dispersal ability of *N. ocellatus* (Atkins *et al.*, 2007), gene-flow is likely to be negligible (see Cliff *et al.*, 2015 for evidence of population genetic isolation). As in the univariate models above, we fit these models with heterogeneous, by-year, error variances. The residual variation was modelled as 16 (year-specific for each population) 2x2 unstructured matrices with covariances constrained to be zero. Residual variance in these models was, therefore, the same as for the univariate models, above. To compare among-individual variance between populations, we used a likelihood ratio test to compare models in which we optimised the likelihood with the variance components for the two populations constrained to be equal to one in which they were unconstrained (see Husby *et al.*, 2010).

Heritability estimates

To estimate the heritable component of variation in reaction norms we incorporated pedigree information into our analysis. To do this, we constructed a pedigree for each population from known maternity records (see Table 1). Paternity is not known for any individuals in either population and could not, therefore, be included in the pedigree. We also ran models with paternity 'dummy coded' by litter to preserve sibship information. Results did not substantially differ when paternity was included in this way (see Supplementary Table S1). We, therefore, present results from models without paternity dummy-coded because, whilst the rate of multiple paternity in *N. ocellatus* is unknown, in closely related species multiple paternity can be very high (Olsson *et al.*, 2005; Uller & Olsson, 2008). Using this pedigree information we were

able to decompose V_I and V_{IxE} into their non-heritable (permanent environmental; V_{PE} and V_{PExE}) and heritable (V_A and V_{GxE}) components. Our model was, therefore:

$$PD_{i,} = XT_i + Z_1\varphi(A_i, n_1, T) + Z_2\varphi(PE_i, n_2, T) + Z_3Yr_i + e_i$$

This model is identical to that presented for the univariate analysis above, except that, Z_1, Z_2 and Z_3 are the design matrices relating to the random effects of the additive genetic (A_i), permanent environment (PE_i), and year (Yr_i) observations, respectively. Thus, $\varphi(A_i, n_1, T)$ is the random regression function of order n_1 of the additive genetic effect of individual i and, similarly, $\varphi(PE_i, n_2, T)$ is the random regression function of order n_2 of the permanent environment effect. As above, models were fit with heterogeneous, by-year residual error variances. Because paternity information was unavailable, and because our pedigree information on mothers was limited, heritable variation in this model is confounded with maternal effects. Consequently, the heritability estimates we present, especially estimates of heritable variation in reaction norm slopes (V_{GxE}), should be treated with some caution and constitute an upper limit on the joint contribution of heritability and maternal effects.

All analyses were performed using ASReml v 4.1 (Gilmore *et al.*, 2015).

Results

Population-level patterns

Over the 16-year field study temperatures differed substantially between the two sites. Mean maximum temperatures during the gestation period varied between 17.4 and 20.8 °C (mean = 19.0 °C) at the lowland site, and between 12.8 and 17.4 °C (mean = 15.58 °C) at the highland site. Consequently, cohort mean parturition dates were later in the highlands than the lowlands. Because of interannual variation in temperatures, however, the range of parturition dates among years was higher in the highlands than the lowlands (Fig. 1). Despite this, the population mean-level reaction norm was similar between sites. At the population level, there was no interaction effect between site and temperature ($F_{(1,20.8)}$ = 0.48, P = 0.495; σ_{Year} = 0.60 ± 0.78 SD; σ_{resid} = 18.34 ± 4.29 SD) or an independent effect of site ($F_{(1,29.3)}$ = 0.0.17, P = 0.685) on cohort-

mean parturition date. There was, however, a significant effect of temperature on mean parturition date ($F_{(1,21.1)}$ = 55.09, P < 0.0001). Thus, temperature had a similar effect on parturition dates at both sites, advancing 5.96 ± 0.81 SD days for each 1°C increase in temperature of (Fig. 1).

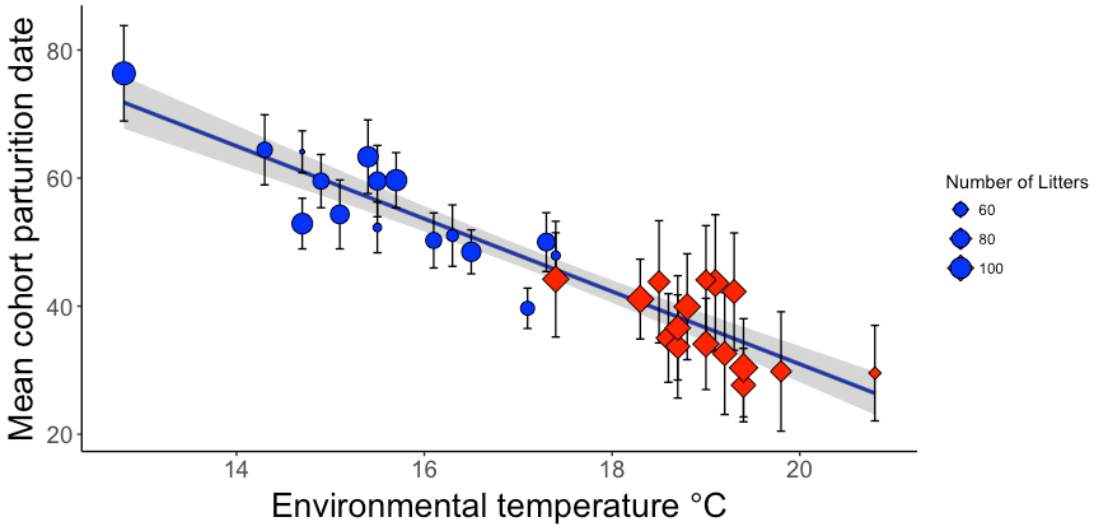

Figure 1: Relationship between mean cohort parturition date and environmental mean maximum daytime temperature during the gestation period in a cold (blue circles) and a warm (red diamonds) population of the spotted snow skink *Niveoscincus ocellatus* between 2000/01 and 2015/16. Error bars show standard deviations from the mean. Point size indicates the number of litters born within years. Parturition dates are given as the number of days after December 10 each year.

Within-population patterns

Univariate models for both populations revealed significant between-individual variation in parturition date (i.e., V_I the intercept of reaction norms) in both populations (P < 0.0001 in both populations: Model 3 in Table 2). In the lowlands we found evidence that individuals varied in the effect that temperature had on parturition dates (i.e, IxE; the slope of reaction norms (plasticity); Model 4 in Table 2 & Figure 2), though this was only marginally significant. In the highland population we found no evidence for among-individual variation in plasticity. In models where both random individual intercepts and slopes were fitted (Model 4, Table 2) the estimated variance of intercepts among individuals in the lowland population was 76.85, compared to 16.44 in the highland population (Table 3). The estimated among

individual variance for reaction norm slope was 1.3 in the lowland population and 0.18 in the highland population (Table 3).

Table 2: Results from site-specific univariate regression analyses of the effect of environmental temperature during the gestational period on parturition dates in a lowland and a highland population of the viviparous skink *Niveoscincus ocellatus* across 16 years. Degrees of freedom, χ^2 values are for comparison with the previous model of log-likelihoods from which *P*-values are calculated. All models were fitted with year-specific error variance (see Methods). V_I is the between-individual variance, which is decomposed into V_{PE} (permanent environmental variance) and V_A (additive genetic variance). IxE is the phenotypic variance-covariance plasticity matrix when no genetic variation in plasticity is fitted. GxE is the additive genetic variance-covariance matrix and PExE is the permanent environment variance-covariance matrix. Variance components of these models are shown in Supplementary tables S2 & S3.

Model	Variance components	*df*	Lowland			Highland		
			LogL	χ^2	*P*-value	LogL	χ^2	*P*-value
1	Null	-	-3952.34	-	-	-2846.18	-	-
2	Year	1	-3815.11	274.46	< 0.0001	-2525.83	640.70	< 0.0001
3	Year + V_I	1	-3664.63	300.96	< 0.0001	-2451.56	148.54	< 0.0001
4	Year + V_I + IxE	2	-3662.00	5.26	0.072	--2450.27	1.29	0.275
5	Year + VA + PE + IxE	1	-3658.09	7.82	0.005	--2450.05	0.22	0.507
6	Year + VA + PE + GxE + PExE	2	-3658.08	0.01	0.990	-2448.65	1.4	0.247

Table 3: Variance components of site-specific univariate random regression models of the effect of environmental temperature during the gestational period on parturition dates in a lowland and highland population of *Niveoscincus ocellatus* across 16 years, fit with random effects for year and individual variation in intercept (V_I) and slope (V_{IxE}) and with heterogeneous by-year error variance (Model 4 from Table 2). Variances are presented ± standard error.

Lowland				Highland			
Residual error				**Residual error**			
2000	10.71 ± 5.15	**2008**	25.31 ± 4.87	**2000**	21.38 ± 8.16	**2008**	22.18 ± 4.73
2001	56.60 ± 11.74	**2009**	22.04 ± 4.63	**2001**	5.83 ± 1.87	**2009**	5.35 ± 2.09
2002	57.05 ± 10.97	**2010**	15.88 ± 3.68	**2002**	44.26 ± 8.02	**2010**	3.69 ± 1.69
2003	67.95 ± 11.36	**2011**	32.03 ± 6.34	**2003**	20.93 ± 4.10	**2011**	11.44 ± 2.89
2004	20.28 ± 4.63	**2012**	44.97 ± 8.98	**2004**	9.43 ± 1.87	**2012**	11.34 ± 2.77
2005	28.52 ± 5.58	**2013**	51.24 ± 10.09	**2005**	7.77 ± 1.84	**2013**	7.05 ± 2.12
2006	28.55 ± 5.68	**2014**	21.07 ± 5.59	**2006**	22.74 ± 3.86	**2014**	11.78 ± 2.50
2007	31.29 ± 7.14	**2015**	1.93 ± 9.18	**2007**	16.68 ± 3.29	**2015**	6.35 ± 2.07
Random effects				**Random effects**			
Year	21.71 ± 8.38			**Year**	21.38 ± 8.16		
IxE variance covariance correlation matrix	76.85	0.93		**IxE variance covariance correlation matrix**	16.44	-0.78	
	9.25	1.30			-1.34	0.18	

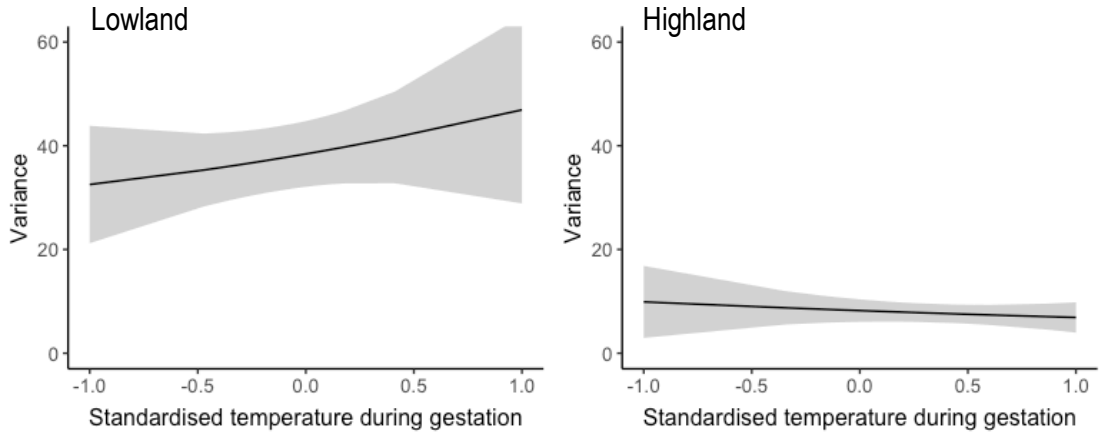

Figure 2: Change in among individual variance (V_I) in parturition date in relation to standardised mean temperature during gestation in a lowland and highland population of *Niveoscincus ocellatus* over 16 years (2000-2015). Shaded areas indicate the approximate 95% confidence interval. Standardised temperatures correspond to mean temperatures on the range 17.4 to 20.8 in the lowland population and 12.8 to 17.4 in the highland population (see Methods).

Between-population comparison

Results from the bivariate models comparing among-individual variation in reaction norms were consistent with the results from the univariate, within-population patterns above. As a test for differences in among-individual variation between the populations we fit a bivariate random regression model, treating parturition date in each population as separate traits with no covariance between them. To test for differences in patterns of variation between the populations we compared a model in which the population-specific variance-covariance matrices were constrained to be equal with a model in which they were unconstrained (see Methods). These tests showed that there was a significant difference between the populations in patterns of among-individual variation in reaction norm intercepts (V_I; $\chi^2_1 = 60.85$, $P < 0.0001$, see Figure 2) and slopes (V_{IxE} $\chi^2_3 = 51.45$, $P < 0.0001$). Thus, this test confirmed substantial differences in among-individual variation in reaction norms between the two populations at the phenotypic level.

Heritability estimates

When we decomposed the variance in reaction norm intercepts among individuals (V_I) within sites into their heritable (V_A) and permanent environment (V_{PE}) variance components (i.e., comparing Models 5 and 4 for lowland and highland populations), we found some support for a heritable component to variation among individuals in our lowland, but not our highland population (Table 2). In contrast, when we decomposed the variance in individuals' response to changes in temperature (i.e., reaction norm slopes; V_{IxE}) into their heritable (V_{GxE}) and permanent environment (V_{PExE}) components (i.e., comparing Models 6 and 5), we found no evidence of a heritable component in either population. Whilst we were unable to detect a heritable basis for variation in mean parturition dates (intercept of reaction norms) in the highlands, or for heritable variation in the effect of temperature (slopes) in the lowlands, the observed variation must arise from an interaction between heritable and environmental sources. Thus, although Model 6 did not provide a significantly better fit than Model 5 for either the highland or lowland population, these models provide the best estimate of heritable and environmental components of the total phenotypic variation for each population and we, therefore present the results derived from these models in Table 4, where we present predictions from Model 6 for each population, using a character state approach.

Table 4: Variance components for parturition date evaluated at standardised temperatures under Model 6 in Table 2. Mean parturition date (μ) is number of days after December 10th. V_P (phenotypic variance) is the sum of variance components, V_A is the additive variance, V_{PE} is the permanent environment variance, V_{Yr} is the year variance, V_R is the residual variance and h^2 is the heritability estimate of parturition date.

Population	Standardised temperature	*n*	μ (SD)	V_P (SE)	V_A (SE)	V_{PE} (SE)	V_{Yr} (SE)	V_R (SE)	h^2
Lowland	-1 (2001)	101	44.22 (0.90)	107.64 (13.15)	17.16 (13.36)	15.71 (12.23)	21.69 (8.41)	53.08 (11.82)	0.16 (0.51)
	0 (2003)	106	43.61 (1.03)	129.35 (9.32)	17.97 (7.52)	20.83 (7.21)	21.69 (8.41)	68.86 (11.50)	0.14 (0.22)
	+1 (2015)	51	29.54 (1.04)	70.85 (6.15)	18.79 (18.02)	30.31 (18.16)	21.69 (8.41)	0.06 (6.33)	0.27 (0.73)
Highland	-1 (2001)	115	76.37 (0.69)	40.9 (10.68)	7.49 (7.05)	6.19 (7.46)	21.38 (8.16)	5.84 (1.87)	0.18 (0.64)
	0 (2006)	87	54.33 (0.58)	46.97 (8.82)	3.21 (2.30)	5.84 (2.37)	21.38 (8.16)	16.54 (3.32)	0.07 (0.32)
	+1 (2009)	49	47.92 (0.51)	33.97 (8.35)	1.85 (2.18)	5.71 (2.54)	21.38 (8.16)	5.03 (2.06)	0.05 (0.46)

Discussion

Because climatically distinct populations within species have faced historically different selective pressures, they are likely to exhibit substantial local adaptation. Thus, it is not clear that populations should necessarily share common phenotypically plastic responses to changes in their climatic environment. Likewise, reaction norms may vary among individuals within populations. Here, we explicitly tested whether patterns of the plastic response (i.e., reactive norms) of parturition date to temperatures experienced during gestation varied within and between climatically distinct populations of the viviparous skink *Niveoscincus ocellatus*. Whilst we found that population-mean reaction norms were similar between highland and lowland populations, we found that among-individual variation was substantially different. We suggest that, because of these differences, populations may respond differently to changing climates, both in the short- and the long-term, even where population-mean reaction norms are similar.

We found that temperature during the gestation period affected parturition dates in both the highlands and the lowlands. However, due to differences in temperatures between sites during this time, parturition occurred later in the highlands than the lowlands. Furthermore, because of differences in the interannual variation of these temperatures, the range of dates over which parturition took place was greater in the highlands across years. Nevertheless, in the highlands and the lowlands, population mean-level reaction norms were not significantly different from each other. We found that parturition dates advanced 5.96 ± 0.84 SE days for each increase in mean maximum daytime temperature of 1°C at both extremes of the climatic range of *N. ocellatus*. Because this mean-level reaction norm is shared between climatically distinct populations, we can project the short-term effect of rising temperatures on cohort mean parturition dates across the full distributional range with some confidence. Mean maximum temperatures in Tasmania are predicted to rise by between 1.6 °C (low emissions scenario) and 2.9 °C (high emissions scenario) over the course of the 21st century and this rise is likely to be relatively uniform across the landscape (Grose *et al.,* 2010). Thus, in the absence of other factors affecting parturition dates, the interannual mean of mean cohort parturition dates can be expected to advance by as much as 17 days by 2100 across the species' range. Even if

we conservatively assume that the variance in temperatures remains similar, mean dates of birth in especially warm years may advance by as much as a full month compared to the current interannual mean (i.e., from mid-January to mid-December and from early February to early January in the lowlands and the highlands, respectively).

Shifts in phenology, such as this, have been widely reported for many species across taxa and are one of the most consistently predicted and observed consequences of warming climates (e.g., Parmesan & Yohe, 2003; Root *et al.*, 2003; Menzel *et al.*, 2006; Parmesan, 2007; Charmantier *et al.*, 2008; Moyes *et al.*, 2011; Lane *et al.*, 2012; Ge *et al.*, 2016; Cohen *et al.*, 2018, Post *et al.*, 2018). Changes in phenology can have positive, neutral or negative effects on individual fitness and population persistence (see Lancaster *et al.*, 2016; Noble *et al.*, 2018). We have previously suggested that, in *N. ocellatus*, advancing parturition dates resulting from warming climates are more likely to have positive than negative effects on population persistence (Chapters 2 & 4: Cunningham *et al.*, 2017; 2018). Indeed, we have shown that earlier dates of birth enhance survival over winter in the lowland population (Wapstra *et al.*, 2010) and, to different degrees, enhance offspring growth (Wapstra *et al.*, 2010; Chapter 4: Cunningham *et al.*, 2018) and result in earlier age and size at maturity (Wapstra *et al.*, 2001; Pen *et al.*, 2010) in both populations. These positive effects are unlikely to be counteracted by factors such as trophic mismatches, because *N. ocellatus* has a generalist diet (Wapstra & Swain, 1996) and is, therefore, able to take advantage of a range of food items that become available at different times during the active season (see Chapter 4: Cunningham *et al*., 2018). However, as climates warm, there is an increasing risk that development may be compromised in extreme years, potentially leading to developmental abnormalities and embryonic mortality. Extreme developmental temperatures have been found to have severe consequences for development across a range of reptilian taxa including turtles (e.g., Telemeco *et al.*, 2013), lizards (e.g., Hare *et al.*, 2002) and snakes (e.g., Lin *et al.*, 2005). Lowland populations may be particularly vulnerable to such effects, as increases in temperature as a result of global climate change are more likely to surpass those populations' critical thermal limits (Caldwell *et al.*, 2015).

Within both highland and lowland populations, individuals varied significantly in the elevation of reaction norms (V_I). We also found evidence that individuals in the lowlands, but not the highlands, varied in the slope of reaction norms (V_{IxE}) though our model showed only marginally significant evidence for variation between individuals. When we compared the variation in reaction norm slopes and intercepts in our bivariate model, we found a significant difference between our populations. Thus, among-individual variation in reaction norms is higher in the lowlands than in the highlands. Significant variation in reaction norm elevations and marginally significant slopes in the lowlands but non-significant variation in reaction norm slopes in the highlands suggests that lowland populations may be at lower risk, in the short-term, from climate warming. Variation between individuals in patterns of plasticity can alter both the mean and variance of phenotypes within populations (Fordyce, 2006). Where all individuals within a population share a common thermal reaction norm of phenology (and, therefore development), all will be similarly affected by extreme events. Thus, if temperatures in extreme years are outside the range of normal development, populations consisting of individuals with similar reaction norms may suffer significant negative consequences, such as malformations and/or increased rates of embryonic mortality (Yntema, 1960; Löwenborg *et al.*, 2011; Telemeco *et al.* 2013). However, in populations where reaction norms vary between individuals, some individuals may not be affected in the same way (Wolf & Weissing, 2012). Thus, whilst populations in the lowlands may be more likely to experience extreme conditions, greater between-individual variation in reaction norm slopes and elevations mean that such events may not have such extreme consequences as they would in the highlands.

In the longer term, changes in environmental conditions are likely to provoke an evolutionary response as selective pressures change. The potential of a population for evolutionary adaptation in a trait, including plasticity itself, is partially determined by the degree to which among-individual variation in that trait has a heritable basis (Matesanz *et al.*, 2010; Franks & Hoffmann, 2012). We found evidence that among-individual variation in elevation of reaction norms has a heritable component in the lowlands, but not in the highlands. Thus, lowland populations may have a greater capacity to respond to changed conditions in the long-term through adaptive evolution. Whilst we found some evidence that individuals in the lowlands differed in

their reaction norm slopes, we did not find evidence that this variation had a heritable component. This lack of evidence for a heritable component of variation in reaction norm elevation in our highland population, and slope in our lowland population, may have resulted from a lack of statistical power to decompose the permanent environmental and heritable components of phenotypic variation. Nevertheless, models with these elements separated (Model 6) provide the best estimate of their relative contributions to phenotypic variation (see Husby *et al.*, 2010). These models suggest that the majority of phenotypic variation in elevation of reaction norms in the highland population, and in slope of reaction norms in the lowland population, arises from non-genetic variation between individuals.

What, might account for these differences between populations? First, because this species is viviparous, observed phenotypes of parturition date in *N. ocellatus* are the result of an interaction of the effects of behavioural thermoregulation and underlying physiology. We have previously shown that pregnant females from the highlands and lowlands alter their basking behaviour to different extents in response to basking opportunities (Uller *et al.* 2011, Caldwell *et al.*, 2017). Thus, it is possible that our failure to detect between-individual variation in the slope of reaction norms in our highland population is the result of underlying variation in physiological plasticity being obscured by compensatory behavioural plasticity. If this is the case, it is probable that this compensation has consequences for female fitness. For instance, less time spent basking may increase the time available for the acquisition of resources or decrease exposure to predators (Kearney *et al.*, 2009).

Second, the lower variation between individuals in the highland population, despite the greater degree of climatic variability at this site, may be the result of a lack of genetic variation in this population compared to the lowland population. A lack of genetic variation in this population may be the result of local adaptation. In the highlands, individuals have a shorter period between parturition and the onset of winter for growth and condition building than those in the lowlands (Atkins *et al.*, 2006; Uller *et al.*, 2010). However, as noted above, we found that earlier dates of birth increase offspring survival at the lowlands, but no such effect in the highlands (see Wapstra *et al.*, 2010 and Chapter 4: Cunningham *et al.,* 2018). Furthermore, if selective pressure on dates of birth arising from time constraints were different

between populations we would expect population-specific mean-level reaction norms (see Edge *et al.*, 2017), rather than similar population-mean reaction norm between sites we found in the present study. We have previously found that increased size at birth enhances survival in the highlands, but not in the lowlands. Thus, canalisation of the developmental reaction norm may have occurred in the highlands because of greater selection for increased mass at birth rather than date of birth *per se* (see Uller *et al.*, 2011).

Third, greater among-individual variation in reaction norms in the lowland population may be the result of greater environmental heterogeneity precluding a canalised response. Indeed, non-heritable variation in the plastic response of parturition dates to temperature at the lowland site may result from fine-scale differences within it. The lowlands and highlands are significantly different from each other, not only in terms of their climate, which is more variable in the highlands than the lowlands, but also in the degree of fine-scale environmental heterogeneity, both within them, and in the surrounding landscape. The lowlands are more heterogeneous than the highlands, with areas of suitable rocky scree overshadowed to different extents by vegetation. Consequently, some areas within the lowland site are more exposed than others and basking opportunities and temperatures vary more among locations within the lowland site and its surrounds than in the highlands. Because of this environmental heterogeneity, and because females show high within-site fidelity, different reaction norms may be optimal among individuals (including between parents and their offspring), depending on their location within the site. Furthermore, optimal reaction norms may differ more among neighbouring lowland populations than is the case for highland populations. This may limit the extent to which reaction norms of individuals in the lowland population may become canalised at an optimum because of gene flow from surrounding populations with different optima (Sultan & Spencer, 2002; Crispo, 2008). In contrast, highland populations are restricted to exposed rocky outcrops without significant vegetation. All individuals within the highland population and its neighbours therefore experience similar conditions and a similar response may be optimal for all individuals within it, facilitating the canalisation of the reaction norm.

We found some evidence for heritable variation in the elevation of reaction norms in our lowland site, but not in our highland site. This implies a greater capacity for the evolution of reaction norms in the lowland, compared to the highland population. Importantly, however, adaptive evolutionary responses may be affected by phenotypic plasticity. Whilst plasticity can promote evolution by enabling population persistence in changed conditions, it can also hinder an evolutionary response by supressing the effects of selection (Ghalambor *et al.*, 2007; Chevin & Lande, 2010; Chevin, 2013; Kovach-Orr and Fussman, 2013). Nevertheless, in *N. ocellatus*, the combination of greater between-individual variation in the elevation and slope of reaction norms at the phenotypic level, and some evidence that the variation in elevation has a heritable component in the lowland population, suggests that lowland populations have a greater capacity to respond to changed climatic conditions in both the short- and the long-term, than highland populations.

Overall, we found that parturition dates in populations of *N. ocellatus* located at the opposite extreme climatic limits of the species' range have a similar population-mean level response to temperature, suggesting that the effect of rising mean temperatures on parturition phenology may be consistent across the species' range in the short-term. Despite this shared population-mean response, however, populations differ substantially in the degree to which the response varies among the individuals within them. Because of these differences among populations, they may be differently affected by extreme events, such as especially warm years leading to developmental stress. Even in the short-term, therefore, populations may be differently affected by changing climates, especially as climatic variation increases and extreme events become more common. In addition, because the level of genetic variation in reaction norms appears to differ among populations, they may also have differing capacities to evolve in response to changed conditions over longer timescales. Thus, even where populations share a common mean-level reaction norm, we should not assume that that they will, necessarily, be affected similarly by altered conditions. If we are to accurately assess how species and populations will be affected by changing climates we require estimates of how mean-level responses vary among populations, and the degree to which responses vary between individuals within populations at the phenotypic, and the genetic, level. As such, long-term studies tracking multiple

populations across generations are of critical, and increasing, importance as climates continue to change.

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Ethics

All guidelines and procedures for the use of animals were approved by the University of Tasmania Animal Ethics Committee (A0017006, A0016736).

References

Atkins, N., Swain, R., Wapstra, E. & Jones, S.M. (2007). Late stage deferal of parturition in the viviparous lizard *Niveoscincus ocellatus* (Gray 1845): implications for offspring quality and survival. *Biological Journal of the Linnean Society*, **90**: 735-746.

Beebee, T.J.C. (1995) Amphibian breeding and climate. *Nature*, **374**: 219-220.

Bolnick, D.I., Amarasekare, P., Araujo, M.S., Burger, R., Levine, J.M., Novak, M., Rudolf, V.H.W., Schreiber, S.J., Urban, M.C. & Vasseur, D.A. (2011). Why intraspecific trait variation matters in community ecology. *Trends in Ecology and Evolution*, **26**: 183-192.

Cadby, C.D., While, G.M., Hobday, A.J., Uller, T. & Wapstra, E. (2010). Multi-scale approach to understanding climate effects on offspring size at birth and date of birth in a reptile. *Integrative Zoology*, **5**, 164-175.

Cadby, C.D., Jones, S.M. & Wapstra, E. (2014). Geographical differences in maternal basking behaviour and offspring growth rate in a climatically widespread viviparous reptile. *Journal of Experimental Biology*, **217**, 1175-1179.

Caldwell, A.J., While, G.M. & Wapstra, E. (2017). Plasticity of thermoregulatory behaviour in response to the thermal environment by widespread and alpine reptile species. *Animal Behaviour*, **132**, 217-227.

Chapman, D.S., Scalone, R., Stefanic, E. & Bullock, J.M. (2017). Mechanistic pecies distribution modelling reveals a niche shift during invasion. *Ecology*, **98**, 1671-1680.

Charmantier, A., McCleery, R.H., Cole, L.R., Perrins, C., Kruuk, L.E.B. & Sheldon, B.C. (2008). Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science*, **320**: 800-803.

Chevin, L.M. & Lande, R. (2010). Adaptation, plasticity and extinction in a changing environment: towards a predictive theory. *PLOS Biology*, **8**, e1000357.

Chevin, L.M., Collins, S. & Lefevre, F. (2013). Phenotypic plasticity and evolutionary demographic responses to climate change: taking theory out to the field. *Functional Ecology*, **27**, 966-979.

Cliff, H.B., Wapstra, E. & Burridge, C.P. (2015). Persistence and dispersal in a Southern Hemisphere glaciated landscape: the phylogeography of the spotted snow skink (*Niveoscincus ocellatus*) in Tasmania. *BMC Evolutionary Biology*, **15**, 121.

Cohen, J.M., Lajeunesse, M.J. & Rohr, J.R. (2018). A global synthesis of animal phenological responses to climate change. *Nature Climate Change*, **8**, 224-228.

Crick, H.Q.P, Dudley, C., Glue, D.E. & Thomson, D.L. (1997) UK birds are laying eggs earlier. *Nature*, **388**: 526.

Crispo, E. (2008) Modifying effects of phenotypic plasticity on interactions among natural selection, adaptation and gene flow. *Journal of Evolutionary Biology*, **21**: 1460-1469.

Cunningham, G.D., While, G.M. & Wapstra, E. (2017). Climate and sex ratio variation in a viviparous lizard. *Biology Letters*, **13**, 20170218.

Cunningham, G.D., Fitzpatrick, L.J., While, G.M. & Wapstra, E. (2018). Plastic rates of development and the effects of thermal extremes on offspring fitness in a viviparous lizard. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, **329**: 262-270. doi: 10.1002/jez.2167

Duputie, A., Rutschmann, A., Ronce, O., Chuine, I. (2015). Phenological plasticity will not help all species adapt to climate change. *Global Change Biology*, **21**, 3062-3073.

Edge, C.B., Rollinson, N., Brooks, R.J., Congdon, J.D., Iverson, J.B., Janzen, F.J. & Litzgus, J.D. (2017). Phenotypic plasticity of nest timing in a post glacial landscape: how do reptiles adapt to seasonal time constrainsts? *Ecology*, **98**, 512-542.

Fordyce, J.A. (2006). The evolutionary consequences of ecological interactions mediated through phenotypic plasticity. *Journal of Experimental Biology*, **209**: 2377-2383.

Franks, S.J. & Hoffmann, A.A. (2012) Genetics of climate change adaptation. *Annual Review of Genetics*, **46**: 185-208.

Ge, Q.S., Wang, H.J., Rutishauser, T. & Dai, J.H. (2016). Phenological responses to climate change in China: a meta-analysis. *Global Change Biology*, **21:** 265-274.

Gilmore, A.R., Gogel, B.J., Cullis, B.R., Welham, S.J. & Thompson, R. (2015). ASReml user guide. Release 4.1 VSN International Ltd, Hemel Hampstead, UK.

Ghalambor, C.K., McKay, J.K., Carroll, S.P. & Reznick, D.N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, **21**: 394-407.

Grose, M.R., Barnes-Keoghan, I., Corney, S.P., White, C.J., Holz, G.K., Bennett, J.B., Gaynor, S.M. & Bindoff, N.L. (2010). *Climate Futures for Tasmania: general climate impacts technical report*, Antarctic Climate & Ecosystems, Cooperative Research Centre, Hobart, Tasmania.

Hare, K.M., Daugherty, C.H. & Cree, A. (2002). Incubation regime affects juvenile morphology and hatching success, but not sex, of the oviparous lizard *Oligosoma suteri* (Lacertilia: Scincidae). *New Zealand Journal of Zoology*, **29**, 221-229.

Henderson, C.R. (1950). Estimation of genetic parameters. *Annals of Mathematical Statistics*, **21**, 309-310.

Husby, A., Nussey, D.H., Visser, M.E., Wilson, A.J., Sheldon, B.C., Kruuk, L.E.B. (2010). Contrasting patterns of phenotypic plasticity in reproductive traits in two great tit (*Parus major*) populations. *Evolution*, **64**, 2221-2237.

Hüppop, O. & Hüppop, K. (2003) North Atlantic oscillation and timing of spring migration in birds. *Proceedings of the Royal Society B – Biological Sciences*, **270**: 233-240.

Jones, S.M., Wapstra, E. & Swain, R. (1997). Asynchronous male and female gonadal cycles and plasma steroid concentrations in a viviparous lizard, *Niveoscincus*

ocellatus (Scincidae), from Tasmania. *General and Comparative Endocrinology*, **108**, 271-281.

Kawecki, T.J. & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, **7**, 1225-1241.

Kawecki, T.J. (2008). Adaptation to marginal habitats. *Annual Review of Ecology, Evolution and Systematics*, **39**, 321-342.

Kearney, M., Shine, R. & Porter, W.P. (2009) The potential for behavioural thermoregulation to buffer "cold-blodded" animals against climate warming. *Proceedings of the National Academy of Sciences of the United States of America*, **106**: 3835-3840.

Kovach-Orr, C. & Fussmann, G.F. (2013). Evolutionary and plastic rescue in multitrophic model communities. *Philosophical Transactions of the Royal Society B – Biological Sciences*, **368**, 20120084.

Kruuk, L.E.B. (2004). Estimating genetic parameters in natural populations using the 'animal model'. *Philosophical Transactions of the Royal Society B – Biological Sciences*, **359**, 873-890.

Lancaster, L.T., Morrison, G. & Fitt, R.N. (2017). Life history trade-offs, the intensity of competition, and coexistence in novel and evolving communities under climate change. *Philosophical Transactions of the Royal Society B – Biological Sciences*, **372**, 20160046.

Lane, J.E., Kruuk, L.E.B., Charmantier, A., Murie, J.O. & Dobson, F.S. (2012). Delayed phenology and reduced fitness associated with climate change in a wild hibernator. *Nature*, **489**: 554-+.

Lin, Z.H., Ji, X., Luo, L.G. & Ma, X.M. (2005). Incubation temperature affects hatching success, embryonic expenditure of energy and hatchling phenotypes of a prolonged egg-retaining snake, *Deinagkistrodon acutus* (Viperidae). *Journal of Thermal Biology*, **30**, 289-297.

Ljungström, G., Wapstra, E. & Olsson, M. (2015) Sand lizard (*Lacerta agilis*) phenology in a warming world. *BMC Evolutionary Biology* **15**, 206.

Löwenborg, K., Shine, R., & Hagman, M. (2011). Fitness disadvantages to disrupted embryogenesis impose selection against suboptimal nest-choice by female grass snakes, Natrix natrix (Colubridae). *Journal of Evolutionary Biology*, **24**, 177-183.

Mägi, M., Semchenko, M., Kalamees, R., Zobel, K. (2011). Limited phenotypic plasticity in range-edge populations: a comparison of co- occurring populations of two Agrimonia species with different geographical distributions. *Plant Biology*, 13, 177–184.

Matesanz, S., Gianoli, E & Valladares, F. (2010). Global climate change and the evolution of phenotypic plasticity in plants. *Annals of the New York Academy of Sciences*, **1206**: 35-55.

McGaugh, S.E., Schwanz, L.E., Bowden, R.M., Gonzalez, J.E. & Janzen, F.J. (2010). Inheritance of nesting behaviour across natural environmental variation in a turtle

with temperature-dependent sex determination. *Proceedings of the Royal Society B – Biological Sciences*, **277**, 1219-1226.

McCaffery, R.M., Maxwell, B.A. (2010). Decreased winter severity increases viability of a montane frog. *Proceedings of the National Academy of Sciences*, **107**, 8644-8649.

Menzel, A. & Fabian, P. (1999) Growing season extended in Europe. *Nature*, **397**: 659.

Menzel, A., Sparks, T.H., Estrella, N. *et al*. (2006). European phenological response to climate change matches the warming pattern. *Global Change Biology*, **12**, 1969–1976.

Mitchell, N.J., Kearney, M.R., Nelson, N.J. & Porter, W.P. (2008). Predicting the fate of a living fossil: how will global warming affect sex determination and hatching phenology in tuatara? *Proceedings of the Royal Society B – Biological Sciences*, **275**, 2185-2193.

Moran, E.V., Hartig, F. & Bell, D.M. (2016). Intraspecific trait variation across scales: implications for understanding global change responses. *Global Change Biology*, **22**: 137-150.

Moyes, K., Nussey, D.H., Clements, M.N., Guinness, F.E., Morris, A., Pemberton, J.M., Kruuk, L.E.B. & Clutton-Brock, T.H. (2011) Advancing breeding phenology in response to environmental change in a wild red deer population. *Global Change Biology*, **17**: 2455-2469.

Noble, D.W., Stenhouse, V. & Schwanz, L.E. (2018). Developmental temperatures and phenotypic plasticity in reptiles: a systematic review and meta-analysis. *Biological Reviews*, **9**, 72-97.

Olsson, M., Ujvari, B., Wapstra, E., Madsen, T., Shine, R. & Bensch, S. (2005). Does mate guarding prevent rival mating in snow skink lizards? A test using AFLP. *Herpetologica*, **61,** 389-394.

Parmesan, C. (2007). Influences of species, latitudes and methodologies on estimates of phenological response to global warming. *Global Change Biology*, **13**, 1860–1872.

Parmesan, C. & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, **421**, 37–42.

Pen, I., Uller, T., Feldmeyer, B., Harts, A., While, G.M. & Wapstra, E. (2010). Climate-driven population divergence in sex-determining systems. *Nature*, **468**, 436.

Phillimore, A.B., Stålhandske, S., Smithers, R.J. & Bernard, R. (2012). Dissecting the contributions of plasticity and local adaptation to the phenology of a butterfly and its host plants. *The American Naturalist*, **180**, 655-670.

Porlier, M., Charmentier, A., Bourgault, P., Perret, P., Blondel, J., & Garant, D. (2012). Variation in phenotypic plasticity and selection patterns in blue tit breeding time: between- and within- population comparisons. *Journal of Animal Ecology*, **81**, 1041-1051.

Post, E., Steinman, B.A. & Mann, M.E. (2018). Acceleration of phenological advance and warming with latitude over the past century. *Scientific Reports*, **8**, 3927.

R Core Team (2014). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.

Root. T.L., Price, J.T., Hall, K.R., Schneider, S.H., Rosenzweig, C. & Pounds, J.A. (2003). Fingerprints of global warming on wild animals and plants. *Nature*, **421**, 57–60.

Rutschmann, A., Miles, D.B., Le Galliard, J.F., Richard, M., Moulherat, S., Sinervo, B. & Clobert, J. (2016). Climate and habitat interact to shape the thermal reaction norms of breeding phenology across lizard populations. *Journal of Animal Ecology*, **85**, 457-466.

Sparks, T.H., Roy, D.B. & Dennis, R.L.H. (2005) The influence of temperature on migration of Lepidoptera into Britain. *Global Change Biology*, **11**: 507-514.

Sultan, S.E. & Spencer, H.G. (2002). Metapopulation structure favours plasticity over local adaptation. *American Naturalist*, **160**: 271-283.

Telemeco, R.S., Warner, D.A., Reida, M.K. & Janzen, F.J. (2013). Extreme developmental temperatures result in morphological abnormalities in painted turtles (*Chrysemys picta*): a climate change perspective. *Integrative Zoology*, **8**, 197-208.

Thackeray, S.J., Henrys, P.A., Hemming, D. *et al.* (2016). Phenological sensitivity to climate across taxa and trophic levels. *Nature*, **535**, 241-245.

Uller, T. & Olsson, M. (2008). Multiple paternity in reptiles: pattern and processes. *Molecular Ecology*, **17**, 2566-2580.

Uller, T., While, G.M., Cadby, C.D., Harts, A., O'Connor, K., Pen, I. & Wapstra, E. (2011). Altitudinal divergence in maternal thermoregulatory behaviour may be driven by differences in selection on offspring survival in a viviparous lizard. *Evolution*, **65**, 2313-2324

Valladares, F., Matesanz, S., Guilhaumon, F., Araujo, M.B., Balaguer, L., Benito-Garzon, M., Cornwell, W., Gianoli, E., van Kleunen, M., Naya, D.E. *et al.* (2014). The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecology Letters*. **17**, 1351-1364.

Vicenzi, N., Corbalan, V., Miles, D., Sinervo, B. & Ibarguengoytia, N. (2017). Range increment or range detriment? Predicting potential changes in distribution caused by climate change for the endemic high-Andean lizard *Phymaturus palluma*. *Biological Conservation*, **206**, 151-160.

Visser, M.E. & Both, C. (2005). Shifts in phenology due to global climate change: the need for a yardstick. *Proceedings of the Royal Society of London B: Biological Sciences*, **272**, 2561-2569.

Visser, M.E. & Holleman, L.J.M. (2001) Warmer springs disrupt the synchrony of oak and winter moth phenology. *Proceedings of the Royal Society B – Biological Sciences*, **268**: 289-294.

Wapstra, E. & Swain, R. (1996). Feeding ecology of the Tasmanian spotted skink, *Niveoscincus ocellatus* (Squamata: Scincidae). *Australian Journal of Zoology*, **44**, 205-213.

Wapstra, E., Swain, R., & O'Reilly, J.M. (2001). Geographic variation in age and size at maturity in a small Australian viviparous skink, *Copeia*, **3**, 646-655.

Wapstra, E., Uller, T., Sinn, D.L., Olsson, M., Mazurek, K., Joss, J. & Shine, R. (2009). Climate effects on offspring sex ratio in a viviparous lizard. *Journal of Animal Ecology*, **78**, 84-90.

Wapstra, E., Uller, T., While, G.M., Olsson, M. & Shine, R. (2010). Giving offspring a head start in life: field and experimental evidence for selection on maternal basking behaviour in lizards. *Journal of Evolutionary Biology*, **23**, 651-657.

Willette, D.A., Tucker, J.K. & Janzen, F.J. (2005). Linking climate and physiology at the population level for a key life-history stage of turtles. *Canadian Journal of Zoology*, **83**, 845-850.

While, G.M. & Uller, T. (2014). Quo vadis amphibia? Global warming and breeding phenology in frogs, toads and salamanders. *Ecography*, **37**, 921-929.

Wolf, M. & Weissing, F.J. (2012). Animal personalities: consequences for ecology and evolution. *Trends in Ecology and Evolution*, **27**: 452-461.

Yntema, C. L. (1960). Effects of various temperatures on the embryonic development of *Chelydra serpentina*. *Anatomical Record*, **136**, 305-306.

Chapter 5: Supplementary Information

Table S1: Results from a univariate regression analysis of the effect of environmental temperature during the gestational period on parturition dates in a lowland and a highland population of the viviparous skink *Niveoscincus ocellatus* across 16 years with paternity 'dummy-coded' by-litter. Degrees of freedom, X^2 values are for comparison with the previous model of log-likelihoods from which *P*-values are calculated. All models were fitted with year-specific error variance (see Methods). V_I is the between-individual variance, which is decomposed into V_{PE} (permanent environmental variance) and V_A (additive genetic variance). IxE is the phenotypic variance-covariance plasticity matrix when no genetic variation in plasticity is fitted.

Model	Variance components	*df*	Lowland			Highland		
			LogL	χ^2	*P*-value	LogL	χ^2	*P*-value
1	Null	-	-3952.34	-	-	-2846.18	-	-
2	Year	1	-3815.11	274.46	< 0.0001	-2525.83	640.70	< 0.0001
3	Year + V_I	1	-3664.63	300.96	< 0.0001	-2451.56	148.54	< 0.0001
4	Year + V_I + IxE	2	-3662.00	5.26	0.072	-2450.27	1.29	0.275

Table S2: Variance components for lowland models presented in Table 2. Variances are given ± standard error. VCC matrices are the variance-covariance-correlation matrices for 1st order terms (intercepts, slopes, covariances and correlations; Models 4-6) or the variance components for 0-order terms (intercepts; Models 3 & 5).

LOWLAND						
	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
Year	-	21.93 ± 8.57	21.77 ± 8.44	21.71 ± 8.38	21.68 ± 8.40	21.69 ± 8.41
2000	63.63 ± 9.74	32.80 ± 4.87	13.29 ± 5.13	10.71 ± 5.15	11.20 ± 5.28	11.18 ± 5.27
2001	81.46 ± 11.49	81.67 ± 11.55	49.07 ± 9.74	56.60 ± 11.74	52.98 ± 11.42	53.08 ± 11.82
2002	92.88 ± 13.80	90.53 ± 13.49	57.84 ± 10.89	57.05 ± 10.97	56.37 ± 10.86	56.38 ± 10.86
2003	187.40 ± 25.92	113.80 ± 15.72	68.81 ± 11.41	67.95 ± 11.36	68.93 ± 11.51	68.86 ± 11.50
2004	55.18 ± 8.16	47.75 ± 7.04	19.01 ± 4.56	20.28 ± 4.63	20.08 ± 4.59	20.07 ± 4.58
2005	66.67 ± 8.99	58.86 ± 7.87	29.35 ± 5.48	28.52 ± 5.58	28.35 ± 5.56	28.35 ± 5.56
2006	76.96 ± 11.49	64.92 ± 9.68	27.48 ± 5.62	28.55 ± 5.68	28.25 ± 5.61	28.26 ± 5.61
2007	87.32 ± 14.11	86.95 ± 14.09	34.71 ± 7.13	31.29 ± 7.14	30.36 ± 7.03	30.37 ± 7.05
2008	78.14 ± 10.99	68.38 ± 9.62	24.36 ± 4.77	25.31 ± 4.87	25.53 ± 4.88	25.50 ± 4.88
2009	52.43 ± 7.41	50.78 ± 7.18	21.09 ± 4.45	22.04 ± 4.63	22.81 ± 4.72	22.81 ± 4.73
2010	40.92 ± 5.67	38.81 ± 5.35	15.05 ± 3.48	15.88 ± 3.68	15.27 ± 3.59	15.30 ± 3.59
2011	66.11 ± 9.60	66.31 ± 9.67	31.42 ± 6.33	32.03 ± 6.34	32.21 ± 6.35	32.18 ± 6.35
2012	154.60 ± 23.28	84.03 ± 12.60	47.75 ± 9.24	44.97 ± 8.98	43.82 ± 8.78	43.81 ± 8.78
2013	119.10 ± 18.84	91.11 ± 14.42	49.22 ± 10.03	51.24 ± 10.09	49.87 ± 9.84	49.88 ± 9.86
2014	144.20 ± 24.03	72.07 ± 12.01	21.84 ± 6.17	21.07 ± 5.59	21.12 ± 5.54	21.07 ± 5.55
2015	73.75 ± 15.37	55.59 ± 11.12	8.40 ± 4.42	1.93 ± 9.18	0.14 ± 7.09	0.06 ± 6.33
PE			75.80 ± 6.29	76.85 ± 6.32	37.37 ± 14.43	41.59 ± 14.44
Cov	-	-		9.25 ± 3.66	40.16 ± 13.85	9.82 ± 7.73
PExE				1.30 ± 4.19	9.72 ± 3.56	2.32 ± 7.99
A					2.36 ± 4.21	36.04 ± 15.02
Cov	-	-	-	-		-0.08 ± 7.56
GxE						0.00 ± 0.00

Table S3: Variance components for highland models presented in Table 2. Variances are given ± standard error. VCC matrices are the variance-covariance-correlation matrices for 1st order terms (intercepts, slopes, covariances and correlations; Models 4-6) or the variance components for 0-order terms (intercepts; Models 3 & 5).

Highland						
	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
Year	-	20.28 ± 7.77	21.42 ± 8.17	21.38 ± 8.16	21.38 ± 8.16	21.38 ± 8.16
2000	14.03 ± 2.17	11.78 ± 1.77	5.24 ± 1.69	5.83 ± 1.87	5.79 ± 1.87	5.84 ± 1.87
2001	59.72 ± 8.04	55.53 ± 7.36	47.02 ± 6.96	44.26 ± 8.02	44.34 ± 8.02	37.44 ± 8.11
2002	39.49 ± 6.19	30.98 ± 4.84	21.26 ± 4.13	20.93 ± 4.10	20.94 ± 4.11	20.75 ± 4.08
2003	37.89 ± 5.42	18.58 ± 2.60	9.38 ± 1.88	9.43 ± 1.87	9.43 ± 1.87	9.46 ± 1.86
2004	94.84 ± 13.80	15.61 ± 2.23	8.24 ± 1.78	7.77 ± 1.84	7.78 ± 1.84	7.29 ± 1.85
2005	70.22 ± 10.22	33.11 ± 4.78	22.73 ± 3.85	22.74 ± 3.86	22.70 ± 3.85	22.49 ± 3.83
2006	52.18 ± 8.04	28.92 ± 4.41	17.18 ± 3.29	16.68 ± 3.29	16.74 ± 3.30	16.54 ± 3.32
2007	48.29 ± 8.05	21.06 ± 3.33	14.37 ± 2.95	14.95 ± 3.13	14.90 ± 3.12	14.92 ± 3.17
2008	29.58 ± 5.04	29.95 ± 5.14	23.21 ± 4.63	22.18 ± 4.73	22.19 ± 4.72	20.88 ± 4.69
2009	26.78 ± 5.78	12.53 ± 2.56	4.64 ± 1.50	5.35 ± 2.09	5.34 ± 2.09	5.03 ± 2.06
2010	15.81 ± 3.43	10.69 ± 2.28	4.35 ± 1.55	3.69 ± 1.69	3.72 ± 1.70	2.65 ± 1.50
2011	33.60 ± 6.96	15.61 ± 3.22	11.63 ± 2.92	11.44 ± 2.89	11.45 ± 2.89	11.26 ± 2.82
2012	22.72 ± 4.30	22.99 ± 4.39	10.95 ± 2.71	11.34 ± 2.77	11.33 ± 2.77	11.43 ± 2.76
2013	18.01 ± 3.00	17.28 ± 2.88	8.04 ± 2.03	7.05 ± 2.12	7.08 ± 2.11	5.37 ± 1.98
2014	23.86 ± 4.05	18.45 ± 3.10	11.79 ± 2.51	11.78 ± 2.50	11.78 ± 2.50	11.91 ± 2.49
2015	51.09 ± 9.62	9.94 ± 1.78	6.04 ± 1.89	6.35 ± 2.07	6.31 ± 2.07	5.88 ± 2.04
PE			15.01 ± 1.74	16.44 ± 2.16	15.62 ± 3.95	12.90 ± 4.74
Cov	-	-		-1.34 ± 1.30	-1.27 ± 1.30	-0.37 ± 2.62
PExE				0.18 ± 1.29	0.17 ± 1.32	0.16 ± 2.60
A					0.77 ± 3.33	5.22 ± 4.58
Cov	-	-	-	-		-2.97 ± 2.56
GxE						1.72 ± 2.50

Chapter 6:

Sex in a Warming Climate: Modelling Changing Patterns of Sex Determination Across Climatic Landscapes

Manuscript status: Cunningham, G.D., Schwanz, L.E., While, G.M. & Wapstra, E. ***in prep.***

Abstract:

Among reptiles there are two main systems by which the sex of offspring is determined: Genetic sex determination (GSD) and Temperature-dependent sex determination (TSD). Species with TSD are thought to be at high risk from changing climates because novel environmental conditions may result in strongly skewed sex ratios. Sex determining mechanisms appear highly labile in some groups of reptiles and various conceptual models have been proposed to explain their adaptive significance and transitions between them. How these models translate to patterns of sex determination across climatic landscapes remains, however, largely unaddressed. For this reason we have a poor understanding of how selective pressures for alternative sex determination mechanisms might be altered as climates change. Here, we used an individual-based evolutionary simulation model, parameterised from a 16-year long-term study, to investigate patterns of selection for alternative modes of sex determination in the spotted snow skink *Niveoscincus ocellatus*, a viviparous skink with intraspecific variation in sex determination mechanisms. Using downscaled climate projections, we predicted patterns of selection for sex determination across the present-day and future (2100) climatic landscape of Tasmania. Our findings suggest that patterns of sex determination may be extremely labile in reptile taxa and that patterns of sex determination within species may be constantly shifting over time.

Introduction:

Populations are often faced with novel environmental conditions. This is true whether they experience changing conditions *in situ* or disperse and colonise new areas. In either scenario, adaptations that represent optimal strategies within a population's current climatic context may not remain optimal under novel environmental conditions (Robertson *et al.*, 2013). As conditions change populations may be buffered against the effects of changed conditions via phenotypic plasticity. In the longer term, however, populations will either become extinct or selection will drive an evolutionary response and populations will become adapted to the new conditions. To assess the consequences of a changing climate for populations and species, researchers require conceptual models that explain how traits that affect population growth and persistence are expected to vary across climatic landscapes, and how selective pressures will be affected by altered conditions.

An important determinant of population persistence is the ratio of males to females in the breeding population (Ginsberg & Milnergulland, 1994; Wedekind, 2002; Rankin & Kokko, 2007; Jenouvrier *et al.*, 2010). This, in the absence of other factors such as sex-specific mortality, is primarily determined by the sex ratio of offspring, especially in short-lived species. Theoretical studies suggest that severe sex ratio skews (especially towards males) have the potential to result in rapid population collapse if their effects are not compensated for by other factors (Janzen, 1994; Le Galliard *et al.*, 2005; Schwanz *et al,* 2010a; Boyle *et al.*, 2014; Mitchell *et al.*, 2010; Hays *et al.*, 2017). For instance, Le Galliard *et al.* (2005) showed that male biased adult sex ratios in the common lizard *Lacerta vivipara* were not compensated for by increased emigration or mortality. Rather, they found that female survival, emigration and birth rates dropped due to increased levels of sexual aggression, leading to an 'evolutionary trap' as the male-skew of the adult sex ratio was amplified. While male skewed sex ratios in the breeding population are a particular concern, extreme female-skewed sex ratios may also be a concern if the proportion of males is reduced to the extent that females fail to mate (i.e, sperm limitation; Rankin & Kokko, 2007).

In most animal species sex is determined either at conception by genes (genetic sex determination; GSD) or after conception by the environment experienced at some later stage (environmental-sensitive sex determination; ESD). A common

form of ESD in vertebrates is temperature-dependent sex determination (TSD), where the temperature experienced during embryonic development determines offspring sex. Species with TSD are thought to be at particular risk from changing climates (Janzen, 1994; Walther *et al.*, 2002) because sex ratios have the potential to become highly skewed as climates diverge from historical patterns to which populations are adapted (Schwanz & Janzen, 2008; Mitchell & Janzen, 2010; Schwanz *et al.* 2010a; Boyle *et al.*, 2014). Understanding the climatic drivers of the evolution of sex determining systems, the transitions between them and how patterns of selection might be altered as climates warm and become more variable has, therefore, never been more important.

Amongst reptiles, sex-determining mechanisms appear to be being highly conserved in some groups (e.g., crocodiles and snakes) but remarkably labile in others (e.g., lizards) and different mechanisms have been reported even amongst closely related species (The Tree of Sex consortium, 2014). Whilst few species show intraspecific variation in sex determining mechanism, those that do (e.g., Conover, 1984; Holleley *et al.*, 2015; Chapter 2: Cunningham *et al.*, 2017) provide an outstanding opportunity to investigate the selective pressures that lead to transitions between systems. Whilst various conceptual models have been proposed to explain transitions between sex determination systems (e.g., Pen *et al.*, 2010; Schwanz *et al.,* 2013) and the selective pressures that have led to the evolution of different patterns among taxa (Charnov & Bull, 1977; Shine, 1999; Pen *et al.*, 2010; Schwanz *et al.*, 2016), how these models translate to patterns of sex determination across climatic landscapes remains largely unaddressed. Consequently, we have a poor understanding of how patterns of sex determining system might be affected as environmental temperatures rise and their variability increases. Understanding patterns of sex determination will allow us to better comprehend the selective pressures that have driven transitions between sex determining systems and to predict how they might be affected by climate change.

Here, we addressed these challenges by using an individual-based evolutionary simulation model parameterised with data from a 16-year dataset tracking births in two climatically-distinct populations of *Niveoscincus ocellatus*, a viviparous skink with intraspecific divergence in sex determining system (Pen *et al*,

2010, Chapter 2: Cunningham *et al.* 2017). In the lowland population, offspring sex is affected by temperature, with a skew towards females (relative to the long-term sex ratio, which is male biased) in warm years and towards males in cool years (Wapstra *et al*., 2009; Chapter 3: Gruber *et al.*, 2018). In contrast, in the highland population, offspring sex is unaffected by temperature and offspring sex ratios vary little between years (Pen *et al.*, 2010; Chapter 2: Cunningham *et al.*, 2017). We have previously modelled the evolution of sex determination patterns in these populations and have shown that they result from sex-specific climatic effects on maturation and the magnitude of between-year climate variability (Pen *et al.*, 2010; details in *The conceptual model*, below). This model successfully explains the evolution of divergent sex determination systems in the climates experienced by the two long-term study populations, but cannot be applied in other climatic contexts. In this study we had three primary aims: 1) To develop an extended model, parameterised with data from our long-term study, to estimate the shape of evolved reaction norms of populations across known climatic parameter space. 2) To estimate the present-day distribution of sex determination systems and the magnitude of cohort sex skews across the range of the species. 3) To predict how selective patterns may be altered by projected changes in climate (2080-2100).

Materials and Methods:

The long-term study system

The spotted snow skink, *Niveoscincus ocellatus*, is a small, (3-10 g, 60-75 mm snout-vent length (SVL)) climatically- and altitudinally-widespread viviparous lizard, endemic to Tasmania, Australia. For the past 16 years (2000/2001 – 2015/2016), births in two populations, located at the extreme climatic limits of the species' range, have been monitored annually. The warm, relatively stable lowland site is located on Tasmania's east coast (42°55' S, 147°53' E, elevation: 30 m) and the cold highland site is located at Lake Augusta on the Central Plateau (41°86' S, 146°53' E, elevation: 1150 m). Climatically these sites differ in both the mean and interannual variability of temperatures experienced by females during the gestational period (here considered as October 1st – December 31st). Specifically, the lowland site is characterised by

relatively higher mean temperatures and lower variability in these temperatures than the highland site (19.00 °C ± 0.7 SD and 14.48 °C ± 1.0 SD at the lowland and highland sites, respectively).

Throughout the long-term study we have followed a standard field and laboratory protocol (see Wapstra & O'Reilly, 2001; Wapstra *et al.,* 2009; Uller *et al.*, 2011; Cadby *et al.*, 2014). Briefly, each year the majority (90-95%) of all pregnant females from each population are caught towards the end of gestation. These females are then returned to a facility at the University of Tasmania, where females are maintained until birth. Following birth, mother and offspring morphometrics (mass; ± 0.1 mg) and snout-vent-length (SVL; ± 0.01 mm) are recorded, and offspring are sexed by hemipene eversion and toe-clipped for future identification. This long-term dataset now includes over 1000 litters and more than 3000 offspring from each population (Supplementary Table S1). Climate variables of our long-term sites were calculated from Australian Bureau of Meteorology (BOM) data obtained via the BOM website, from weather stations located close to our study sites. The long-term mean (T_m) and standard deviation (SD_T) in annual temperatures at these sites were calculated as the mean maximum temperature from October 1st to December 31st and the standard deviation in these for the 20-year period 1996 to 2016.

The conceptual model

We have previously developed a conceptual model to explain the differences between our two long-term study populations in the response of offspring sex to temperature in *N. ocellatus*, as adaptations to local climate (Pen *et al.* 2010; Figure 1). This model showed that divergent sex determination has evolved due to a combination of population-specific differences in the sex-specific fitness effects of date of birth (which is affected by temperature) and differences between the two populations in the interannual variation of temperatures. Ovulation is highly synchronised, at both sites, and is consistent among years (Wapstra *et al.*, 1999). Development rates and birth dates are affected by environmental temperatures experienced during gestation, such that births occur earlier in warm years and later in cold years (Uller *et al.*, 2011; Chapter 2: Cunningham *et al.*, 2017). Because temperatures are lower at the highland site than the lowland site, birth dates are later and are more synchronised (Wapstra *et*

al., 1999; Pen *et al.*, 2010; Chapter 2: Cunningham *et al.*, 2017; Chapter 5). As a result, at the highland site, date of birth has a comparatively weak effect on the age at which females reach maturity and, therefore, on lifetime reproductive output (Pen *et al.*, 2010; see Figure 3 and Supplementary Tables S2 & S3). In contrast, at the lowland site, early born females have substantially higher lifetime reproductive success than late-born females, because they have a longer period for growth and condition building in their first year and thus mature earlier (see Figure 3 and Supplementary Tables S2 & S3). Date of birth does not affect reproductive success for males at either site (Pen *et al.* 2010).

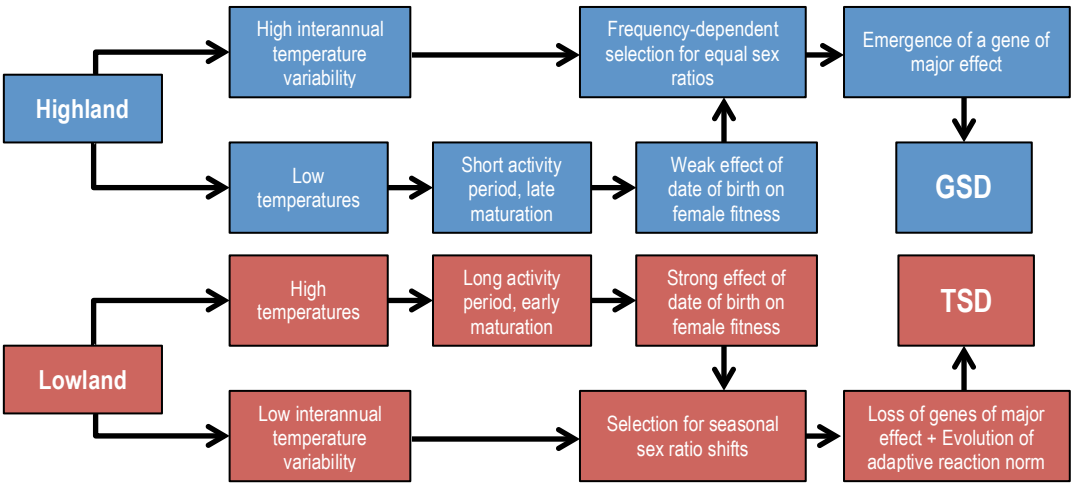

Figure 1: Flow chart describing the climatic, physiological and evolutionary processes underlying population divergence in sex-determining systems in the snow skink, *Niveoscincus ocellatus* (from Pen *et al.*, 2010).

These differences in the sex-specific effects of birth date on fitness between the two populations have resulted in selection for a mechanism linking sex to temperature (i.e., TSD) in the lowland, but not the highland population. Additionally, the comparatively high interannual variability in temperatures at the highland site mean that a sex response to temperature in this population would result in maladaptive highly skewed cohorts in some years. As a consequence, GSD is favoured at the highland site as a mechanism to ensure equal sex ratios (i.e., frequency-dependent selection on sex (Van Dooren and Leimar, 2003; Schwanz and Proulx, 2008; Bull & Bulmer, 1989)). Mean temperatures and their interannual

variation, thus, affect selection on a sex response to temperature differently. Long-term mean temperatures determine the strength of selection favouring a sex ratio response to temperature, whilst variation in these temperatures affects the strength of selection against a sex ratio response (Figure 1). This model successfully established an adaptive explanation for divergence in sex determination system and explains observed differences in the effect of temperature on offspring sex as optimal strategies in two specific climatic contexts. Thus, it provides a powerful explanatory framework integrating developmental and ecological parameters to explain the evolution of transitions between sex determination systems. However, this conceptual model, because it is specific to these two environmental contexts, has not been applied to climatic scenarios other than those experienced by our long-term populations (i.e., cold-variable or warm-stable). Thus, we have no understanding of how this conceptual model plays out across climatic landscapes.

Simulation Model overview (details below)

To investigate how sex determination patterns might vary across present-day and future climatic landscapes, we extended Pen *et al.*'s conceptual model, using an individual-based evolutionary simulation model (Figure 2), parameterised with data from our 16-year field study. Briefly (details below), we performed 400 replicate simulations, each representing a population located randomly within a theoretical climatic parameter space of possible climates across Tasmania (see *Climate*, below). Population climate, in each replicate simulation, was defined by 1) a long-term mean and 2) a standard deviation of a normal distribution, of yearly mean maximum daytime temperature during the gestation period (October 1st – December 31st). Yearly temperatures were sampled from these parameters and defined the cohort mean date of birth. Variation in date of birth was linked to the long-term mean temperature because our long-term data show that colder sites have lower variation in birth dates than warmer sites (see *Lifecycle*, below). Offspring date of birth was sampled from mean and variance in date of birth.

The date of birth of each offspring determined its "effective developmental temperature" representing the actual thermal environment offspring experienced *in utero*. This developmental temperature was used to assign offspring sex according to

a threshold polymorphism mechanism involving four gene loci (see *Sex determination and genetics*, below) proposed by Quinn *et al.* (2007). In the Quinn *et al.* (2007) model, each individual has a temperature-dependent gene product dosage determined by genetics. If this dosage exceeds a genetically determined threshold, an individual develops as a male (see Figure 4). This framework can represent TSD, GSD, and sex reversal systems. To provide the opportunity for the transitions between sex determining systems in our simulations, we allowed alleles at these gene loci, other than those corresponding to sex chromosome alleles, to mutate in each generation (see *Sex determination and genetics*, below).

Females had a probability of maturing early, dependent on their date of birth and on the long-term mean temperature of the replicate simulation, representing the effect of length of the activity season. Thus, date of birth affected the probability for females, but not males, of reaching maturity early (see *Lifecycle*, below). At the beginning of all replicate simulations, populations were represented by an XY GSD system. Replicate simulations were run for 50,000 simulated years, the last 20 of which were used to determine whether the population had evolved TSD (defined here as a significant relationship between yearly temperature and cohort sex ratio over the last 20 years of replicate simulations; see *Sex determination across parameter space*, below). In this model, when mean and variation of yearly temperatures produce sufficient variation in date of birth to impact probability of early maturation in females, we predicted that the sex determination system would evolve such that early birth date (and thus, warm temperatures) becomes associated with development into females (i.e., TSD).

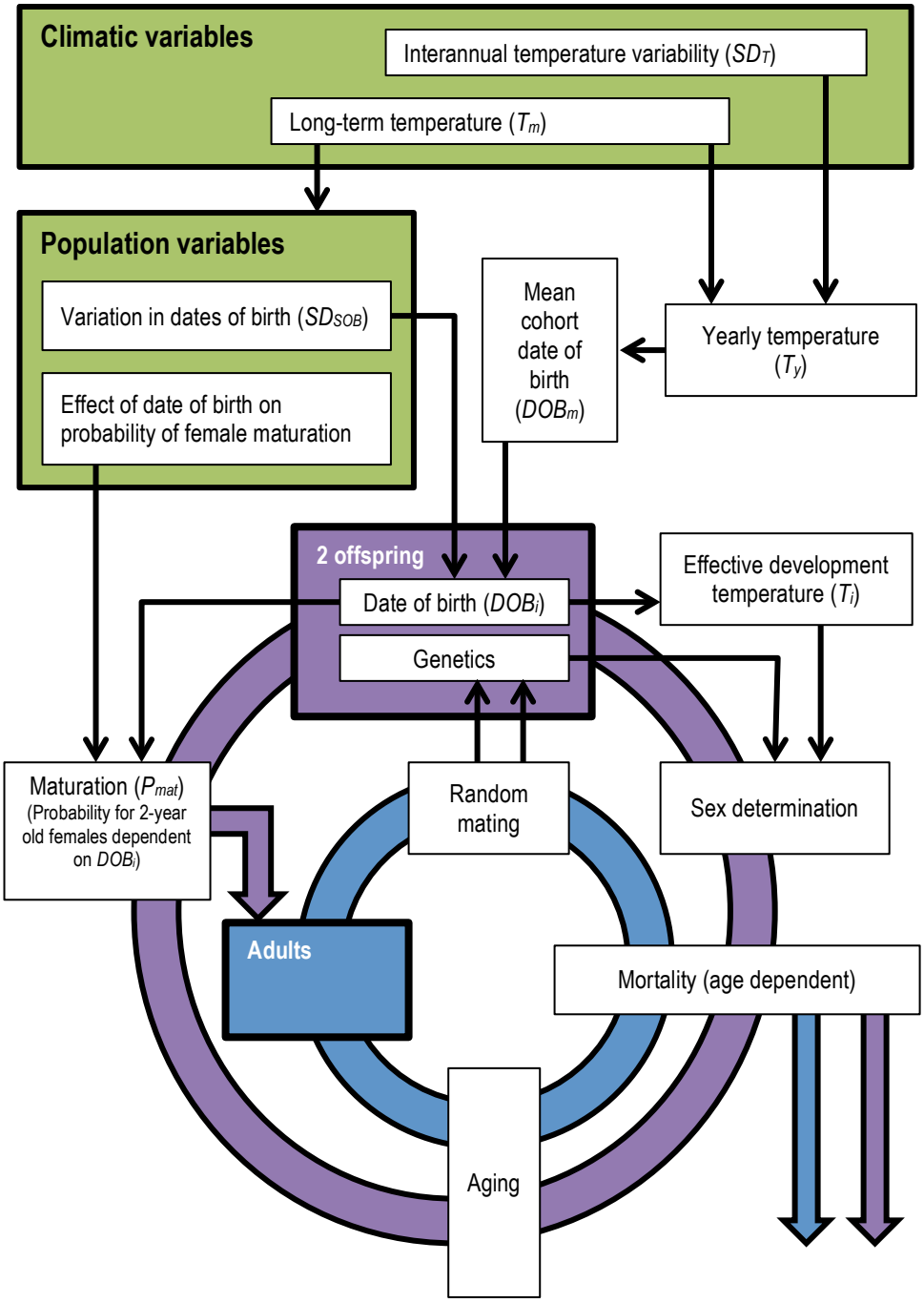

Figure 2: Flow chart showing the general sequence of replicate simulations. Replicate-specific variable are shown in green boxes, adult life-cycle is shown in blue and juvenile life-cycle is shown in purple. Coefficients are shown in Supplementary Table S4.

Climate

In each of 400 replicate simulation populations, climate was described by two parameters: 1) the long-term mean (T_m) and 2) the standard deviation (SD_T) of the mean maximum daytime temperature over the gestational period (October 1st – December 31st). Thus, in this model, climate can be visualised as a location in a two-dimensional climatic parameter space representing possible climates across Tasmania. Together, these parameters determined the mean maximum daytime temperature over the gestational period in each year (T_y) of replicate simulations randomly according to a normal distribution. Within each replicate simulation the values of T_m and SD_T for the population were selected from a uniform distribution of ranges 10 – 25 °C (T_m) and 0.2 to 2.0 (SD_T). These ranges include the full range of climates (as defined by these variables) currently experienced within *N. ocellatus'* distribution and likely changes as climates warm and become more variable over the next century (see Figure 8). In addition to determining yearly temperature, the long-term mean temperature (T_m) was also used to determine two relationships that differ between populations in our long-term study: 1) the within-year variation in litter birth dates (SD_{DOB}) and 2) the relationship between birth date and the probability (M_S) of females maturing early (details in *Life Cycle*, below). Thus, in our model, warmer overall climate leads to longer breeding and growing seasons, as we have observed empirically in our long-term study. The relationship between T_m and each of these variables was estimated from our long-term data, assuming a linear relationship between their values in our long-term populations (see Supplementary Table S4).

Life Cycle

For each year in a simulation, a mean date of birth of litters (DOB_m) was calculated from the yearly temperature (T_y) according to a linear relationship that was the same for all simulation replicates. This relationship was derived from our long-term study (see Supplementary Figure S1; Supplementary Table S4) and represents the reaction norm of temperature on developmental rates, such that the mean date of birth was earlier in warmer years than in cooler years. Each year, all mature females were mated with a random mature male to produce a litter of two offspring. Some males, therefore, mated multiply, whilst others remained unmated. Both offspring were

assigned the same date of birth (DOB_i), which was randomly assigned from a normal distribution of mean DOB_m and standard deviation SD_{DOB}. Because the variation in birthdates in our long-term study depends on interannual climate, SD_{DOB} was calculated from the long-term mean temperature of the replicate simulation (T_m) according to a linear relationship parameterised with data from our long-term dataset (Supplementary Table S4). SD_{DOB} was higher in simulations with low mean temperatures than in simulations with high mean temperatures. Thus, the spread of birth dates within cohorts differed amongst simulation replicates, increasing as long-term mean temperature of the simulation replicate (T_m) rose.

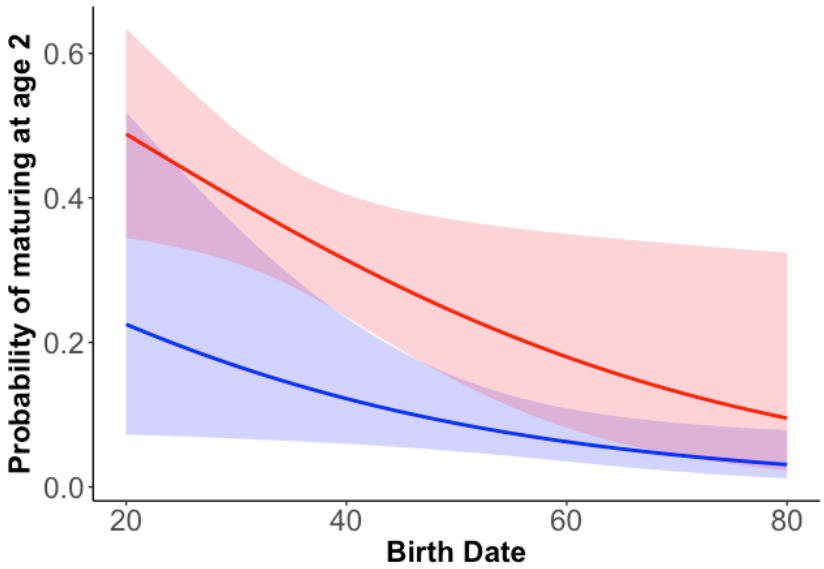

Figure 3: Effect of date of birth on the probability of female *N. ocellatus* maturing at age 2 in the simulation model dependent on the long-term mean annual temperature (T_m) of the simulation replicate. Coefficients from a GLM of this effect at highland (blue) and lowland (red) sites over 16 years (2000-2001) in the field were used to parameterise the curve and effect of T_m assuming a linear relationship (see Supplementary Table S3).

Sex determination depends on developmental temperature; however, actual developmental temperatures and their relationship to climate are not known in our long-term studies. To represent the temperature experienced by each offspring during development, its date of birth (DOB_i) was used to determine an "effective developmental temperature" (T_i). Each individual's T_i was calculated from DOB_i using the same relationship used to establish the cohort mean date of birth from yearly

temperature. Thus, individuals born on the mean cohort date of birth (DOB_m) had a T_i equal to the yearly temperature (T_y), whilst those born later had a lower T_i and those earlier a higher T_i. While this developmental temperature is arbitrary, the temperatures of the sex determining system are also arbitrary; the important feature is how they relate to one another. Because dates of birth varied more between litters as long-term mean temperatures rose, the variability in effective development temperatures also rose with increasing long-term mean temperatures. At conception, an individual's T_i, in combination with its genetically determined signal dosage curve, was used to determine its sex (see *Sex determination and genetics*, below).

Following reproduction, individuals between 1 and 10 years survived with a constant probability of 0.7, in line with data from skeletochronology and our long-term data (Wapstra *et al.*, 2001; Pen *et al.*, 2010). Survivors were aged by one year. Survival of newborn offspring to age one was density-dependent. At age two, females, but not males, had a probability (P_{mat}) of maturing (Figure 3). The probability of each female maturing at age two was determined by its date of birth (DOB_i) and a maturation effect (M_s), based on the long-term climate (T_m) to capture the effect of the length of the active season and temperature-dependent rates of growth on the probability of females maturing early. We parameterised this effect using our long-term data by fitting a binomial GLM (logit link) to the probability of females maturing at age two with date of birth and site as fixed factors. We estimated M_s by assuming a linear relationship between long-term mean temperature and the β-coefficient of the effect of site from this model (Supplementary Table S3 & S4). Thus, the relationship between date of birth and M_s was the same for all individuals within a replicate simulation. The probability of maturing at age two for individuals born on the same date in simulation replicates representing colder sites (i.e., lower T_m) was lower than those in replicate simulations representing warmer sites (i.e., higher T_m). At age three, all immature females and males became mature. Thus, within replicate simulations, females born early had an increased chance of maturing at age two compared to males and later-born females (Figure 3). The initial population in replicate simulations consisted of 2000 mature individuals, all two years old and sexed according to randomly allocated genes (see *Sex determination and genetics*, below).

Sex determination and genetics

Following Pen *et al.* (2010), we modelled sex determination as a threshold polymorphism, based on a mechanistic model by Quinn *et al.* (2007). In this model (Figure 4A) individuals are genetically defined by three diploid gene loci (μ, σ and κ), which determine a signal dosage curve across development temperatures and a fourth locus (τ), which determines a male-determining signal dosage threshold, above which individuals develop as males, and below which they develop as females. In this model, the signal dosage curve is defined as a normal curve with a peak at an effective development temperature (T_i) at the mean value (μ_i) of alleles at an individual's μ locus, and spread determined by the mean value (σ_i) of alleles at its σ locus. To determine signal expression (δ_i), signal dosage at an individual's a effective development temperature (T_i) is multiplied by the mean value (κ_i) of two alleles corresponding to a locus on a sex chromosome (κ), one of which has a high level of expression (κ_A = 1.00), and the other a low level of expression (κ_a = 0.25). $\kappa_A\kappa_A$ individuals, therefore have a κ_i=1, $\kappa_a\kappa_a$ individuals a κ_i=0.25 and $\kappa_A\kappa_a$ individuals a κ_i =0.625. An individual's male-determining signal dosage threshold (τ_i) was defined by the mean values of alleles at its τ locus. This model can represent male heterogametic (XX/XY), female heterogametic (ZZ/ZW) or TSD systems. For example, an XX/XY system can be represented as one in which all heterozygous individuals ($\kappa_A\kappa_a$) have a signal dosage that exceeds their threshold ($\delta_i > \tau_i$), and thus develop as males across the range of development temperatures actually experienced, whilst all homozygous individuals ($\kappa_a\kappa_a$) have a signal dosage below their threshold ($\delta_i < \tau_i$), and thus develop as females across these temperatures (Figure 4B). Similarly, a ZZ/ZW system can be represented as one in which all $\kappa_A\kappa_A$ individuals have a signal dosage above their threshold whilst all $\kappa_A\kappa_a$ individuals have a signal dosage below their threshold. A TSD system, meanwhile, can be represented as one in which all individuals are homozygous for κ, and whether an individual's signal dosage exceeds its threshold depends on its development temperature (Figure 4C) which, in turn depends on the yearly environmental temperature. Sex reversal (where an offspring's phenotypic sex does not match its karyotype) can, likewise, be represented as one in which whether an individual develops as male or female generally depends on its karyotype, but in which some karyotypes that usually develop as one sex develop as the other sex at temperature extremes (Figure 4D).

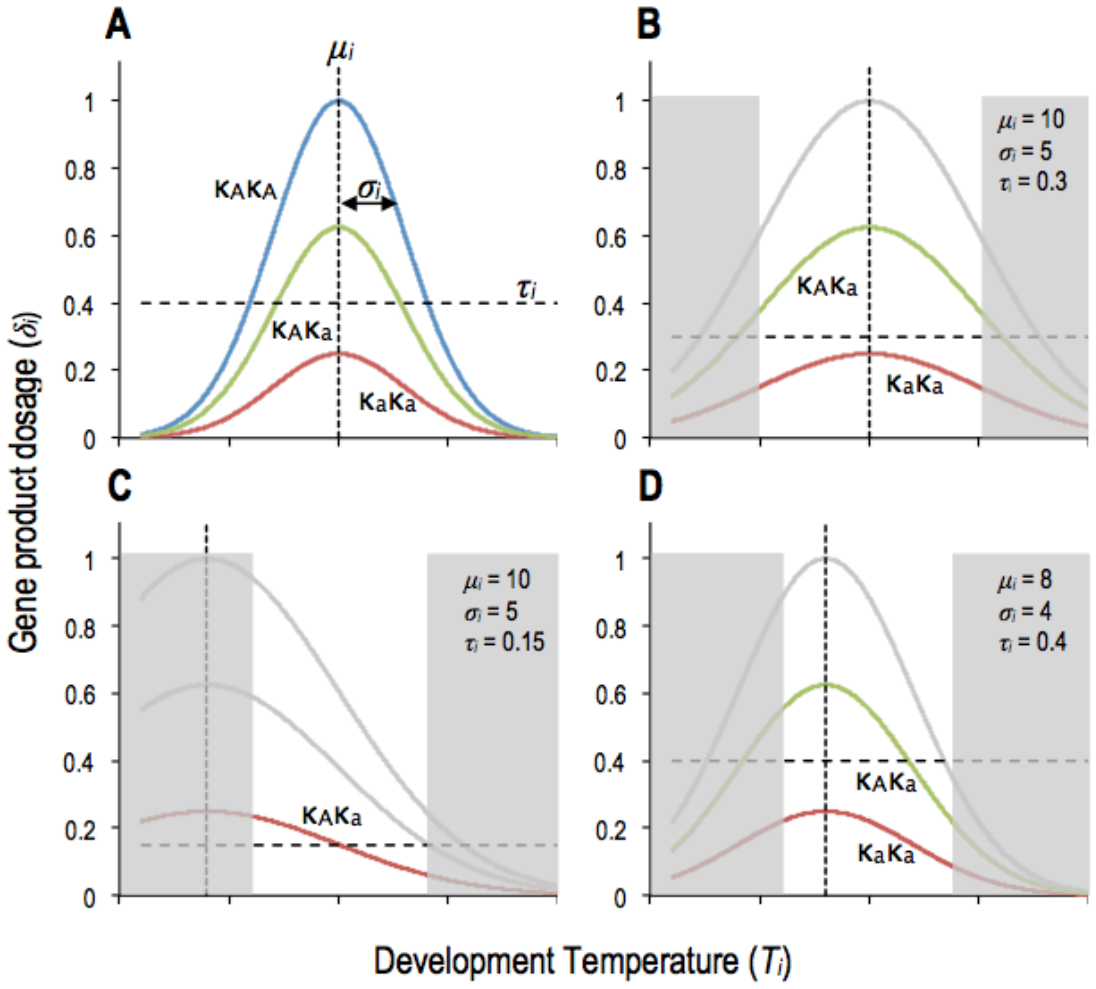

Figure 4: Mechanistic model following Quinn *et al.* (2007). Sex is determined such that individuals develop as males if a gene product dosage exceeds a genetically-determined threshold (τ_i) and as females if the gene dosage is below this threshold. An individual's gene dosage is defined from a normal curve with a genetically determined peak (μ_i) and spread (σ_i) across developmental temperatures (unshaded area represents range of temperatures actually experienced) multiplied by the mean effect of sex chromosome alleles (κ_A and κ_a). This mechanism can represent **B)** a GSD system (in this case XX/XY), **C)** a TSD system (in this case females at high temperatures and males at low temperatures, or **D)** or a GSD system with sex reversal at extreme temperatures (in this case an XX/XY system with sex reversed XY females at high temperatures).

Based on genetic data from our two long-term populations, *N. ocellatus* has an ancestral XX/XY sex determination system (Hill *et al.*, 2018). Each individual in the initial population was, therefore, randomly assigned one of two κ karyotypes: $\kappa_A\kappa_a$ or $\kappa_a\kappa_a$, and sex was assigned, such that $\kappa_A\kappa_a$ individuals were male and $\kappa_a\kappa_a$ individuals female. All individuals were assigned two identical τ, μ and σ alleles and were, therefore, genetically identical in the initial population, except that females were homozygous for κ whilst males were heterozygous. Offspring randomly inherited a single κ, μ, σ and τ allele from each parent, each of which had a 0.1 probability of mutating to a new value from a normal distribution with a mean equal to its current value and a standard deviation shown in Table 1. Sex chromosome (κ) alleles did not mutate. κ_A and κ_a, values, therefore, remained constant across years. To ensure that sex reversal did not occur within the first 50 generations of any simulation (i.e., a stable GSD system) in the initial population all τ alleles were assigned a value of 0.275, μ alleles a value equal to the long-term mean temperature (T_m), and σ alleles a value scaled (Table 1) according to the standard deviation in temperatures between years (SD_T) and the standard deviation in dates of birth within years (SD_{DOB}).

Table 1: The initial values, probability of mutating in each generation and standard deviation of mutation of alleles determining gene dosage across developmental temperatures. In each generation each allele had a chance of mutating to a new value according to a normal distribution with a mean at its current value and its corresponding standard deviation of mutation. Initial values were selected to avoid sex reversal occurring within the first 50 generations of replicate simulations.

Gene locus	Initial value	Mutation probability	Standard deviation of mutation
κ_A	1	0	-
κ_a	0.25	0	-
μ	Long-term mean temperature of replicate simulation (T_m)	0.1	0.05
σ	$2.5 \times SD_T + 2.5 \times \sqrt{((-6.8 + 0.8 \times T_m)/-4.596)^2}$	0.1	0.005
τ	0.275	0.1	0.002

Selection across parameter space

We ran 400 replicate simulations (see above) for 50,000 simulation-years. For each of these simulation replicates, we estimated the relationship between (mean-centred) yearly temperature and cohort sex ratio over the last 20 years of each simulation replicate by fitting a binomial GLM (logit link). To predict how selection would shape reaction norms of sex determination across climatic parameter space, we generated a two-dimensional interpolation surface predicting the effect of temperature on offspring sex across climatic parameter space (Selection model, hereafter). To do this we fit a thin-plate spline to the β-coefficients from the GLMs fit to each replicate simulation, using the *tpaps* function in Matlab 2015b, with the default smoothing parameter (p = 0.0387). To predict the degree to which sex ratios would be skewed across the parameter space within the range of yearly temperatures actually experienced, rather than the effect of a unit change in temperature, we scaled the selection model by the standard deviation (SD_T) of yearly temperatures (Scaled selection model, hereafter). Thus, we show models predicating the response of cohort sex ratios across parameter space to a change in annual temperature of both 1 °C (Selection model) and 1 SD in annual temperature (Scaled selection model). We report the range of sex ratios experienced in years with temperatures ± 2 SD from the long-term mean (i.e., the range of temperatures likely to be experienced by populations). To investigate patterns in the genetic mechanisms underpinning sex determination systems, we also defined each simulation replicate population as either TSD or GSD using the GLM models above. We defined simulation replicates as TSD if the model showed a significant effect of (mean centred) yearly temperature on cohort sex ratio at a significance level of $P < 0.000125$ (i.e., a Bonferroni correction for 400 tests), and as GSD if this relationship was non-significant.

Selection across landscapes

Using our selection model we were able to predict the strength of the relationship between yearly temperature and the cohort sex ratio across the current and projected climatic landscape of Tasmania. To do this, we used five climate projection models (*ECHAM 5*, *GFDL CM 2.0*, *GFDL CM 2.1*, *MIROC 3.2* (medres) and *UKHad CM 3*), downscaled to 14 km resolution (Grose *et al.*, 2010) to develop arrays of the present

day (1996-2016), long-term mean (T_m) and standard deviation (SD_T) in the yearly mean maximum daytime temperature during the gestation period (October 1st – December 31st) across Tasmania. We then applied the selection model developed from our simulations (above) to these arrays. Using projections from the five climate models of T_m and SD_T for the period 2080-2100, we were able to use the same process to project how the selective pressures for alternative sex determination systems might be altered over the coming century. Thus, models applied to the current (1996-2015) climatic landscape represent the present-day reaction norms of populations, whilst those applied to the future (2080-2100) represent the direction of selection under altered climatic conditions.

Sex ratios in a warming climate

By applying this landscape model to projections from the five climate projection models for the periods 1996–2006 and 2080-2100, we predicted cohort sex ratios under present day and projected climates across the landscape, assuming no evolution of the modelled present-day reaction norm describing the relationship between yearly temperature and cohort sex ratios. Additionally, we used our selection model and these climate projections to investigate the direction of selection for the shape of reaction norms under altered climatic conditions. All five climate models substantially agreed in their projections of long-term mean temperatures during the gestation period but differed somewhat in their projections of the variation in these temperatures between years (Supplementary Figure S7). Nevertheless, landscape-wide reaction norm patterns derived from the five climate models were substantially the same. We, therefore, present results derived from the mean of the five models' projected values of T_m and SD_T across the landscape, and provide results derived from each separate projection model as supplementary information (Supplementary Figures S7 & S8).

Sensitivity

We tested whether the observed patterns of the relationship between yearly temperature and cohort sex ratios (i.e., reaction norms) in our simulations resulted from the effect of date of birth on the probability of females maturing early, as

hypothesised. To do this we ran 400 simulations with the same parameters as described for the base simulation model described above, except that the probability of females maturing at age two did not vary with date of birth, but was constant at 50% and compared these results to those from our base model. To visualise the relative strength of the effect of date of birth on the probability of females maturing at age two across the range of long-term mean temperatures (T_m) of replicate simulations (i.e., the relative strength of selection for TSD), we calculated the difference in probability of maturation for females born +2 and -2 standard deviations from the mean date of birth in an average year (i.e., when $T_y = T_m$; see Supplementary Figure S3). We also tested the sensitivity of our results to the number of simulation-years over which models were run and to the mutation rate of mutating alleles by 1) running an additional 100 simulations for 100,000 years, and 2) running 100 simulation replicates with a mutation rate of 0.5 per inherited allele. These models resulted in similar overall patterns to those from the base simulation model. We therefore present results from the base model only and provide results from these alternative models in the supplementary information (Supplementary Figures S5 & S6).

Statistical analysis

Analyses of the long-term dataset for model parameterisation were conducted in R (R Core Team, 2017) using the 'lme4' package (Bates *et al.*, 2014) for GLM models. Model simulation, analysis, thin-plate splines and climate mapping were performed in Matlab 2015b (2015).

Results:

Selection across parameter space

Our selection model showed a response of offspring sex to temperature across parameter space defined by long-term mean (T_m) and standard deviation (SD_T) of yearly temperature with an internal peak at approximately 21^{o}C, and a decline in this effect as the interannual variation in yearly temperatures (SD_T) increased (Figure 5). Consistent with our long-term data, this model projected a strong effect of

temperature on offspring sex at the position in parameter space occupied by our lowland (TSD) population and a weak effect at the climatic position occupied by our highland (GSD) population.

The internal peak in the effect of temperature on offspring sex at 21 °C corresponds to the mean long-term temperature at which the difference in probability of maturing at age two between early-born (-2 SD from the mean) females and late-born (+2 SD from the mean) females is at its highest (Supplementary Figure S3). Thus, the selective peak for a strong effect of temperature on offspring sex at 21 °C is due to the sex-specific relative fitness advantage of date of birth peaking at this temperature, resulting in strong selection for a relationship between temperature and offspring sex (i.e., TSD). Furthermore, these results demonstrate that the selection for TSD does not continue to rise as mean temperatures (and therefore lengths of the active seasons) rise, but drops at long-term mean temperatures above 21 °C, despite high-temperature populations exhibiting greater variance in dates of birth. This pattern arises because, at both low and high temperatures, the probability of late and early born females maturing early is similar. Thus, irrespective of date of birth, females in cold climates almost all mature at age three whilst, in warm climates, almost all mature at age two. Thus, date of birth provides no selective advantage in either extreme high or low mean temperature climate scenarios and there is, therefore, no positive selection for TSD (see Supplementary Figure S4).

We found that climate variability also affected selection for GSD as a stabilising mechanism due to frequency-dependent selection on sex. In our unscaled selection model, the strength of the effect of a 1°C change in yearly temperature on offspring sex fell as climatic variation (SD_T) increased. When simulations were run with the probability of females maturing at age two independent of date of birth (i.e., without a sex-specific advantage of date of birth), the strength of the response of sex to a 1°C change in yearly temperature did not substantially vary across parameter space (Supplementary Figure S10). This confirms that the strength of selection for alternative sex determination systems is contingent on the strength of selection for TSD, which depends on mean annual temperatures, and selection for GSD, which depends on the variation in these temperatures.

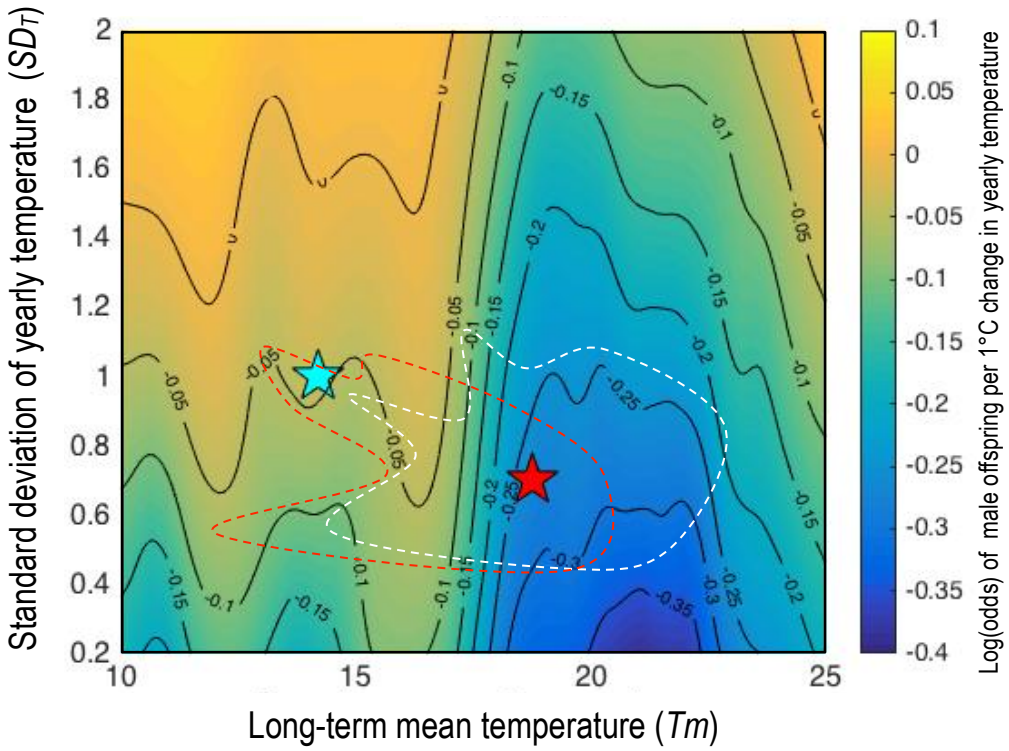

Figure 5: The selection model: derived from a thin plate spline interpolation of the relationship between a 1°C change in yearly temperature and the change in the log(odds) cohort sex ratio of males: females across climatic parameter space defined by mean (T_m) and standard deviation (SD_T) of annual mean temperature derived from 400 simulated populations. Values above 0 indicate a skew towards males at high temperatures, those below 0 a skew towards females at high temperatures. Stars indicate the position in parameter space of the lowland (red) and highland (blue) study sites. Areas enclosed by dashed lines indicate the present day (1996-2016; red) and future (2080 – 2100; white) climatic envelopes across the landscape of Tasmania from the mean of five climate projection models.

To predict the degree of expected sex ratio skews within the range of temperatures likely to be experienced by populations across parameter space we scaled the response of offspring sex to yearly temperature across parameter space by the standard deviation of yearly temperature (scaled selection model; Figure 6). When the selection model was scaled in this way, we found that the strength of expected sex ratio skews across years peaked at mean annual temperatures (T_m) of 21 °C, and where the standard deviation of these temperatures (SD_T) was approximately 1.5. Thus, despite the strength of the response to a 1°C change in yearly temperature declining as SD_T increased (Figure 5), sex ratio skews were predicted to increase in populations with higher interannual variation in temperatures.

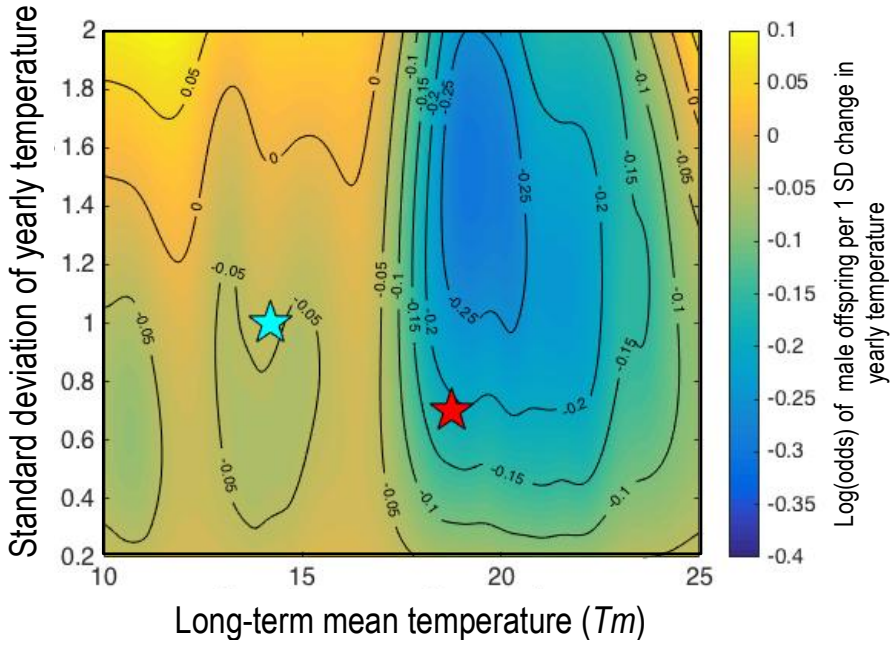

Figure 6: The scaled selection model: derived by scaling the selection model shown in Figure 5 by the standard deviation (SD_T) of annual mean temperature such that the scale indicates the relationship between a 1°C change in yearly temperature and the change in the log(odds) cohort sex ratio of males: females across climatic parameter space. Values above 0 indicate a skew towards males at high temperatures, those below 0 a skew towards females at high temperatures. Stars indicate the position in parameter space of the lowland (red) and highland (blue) long-term study sites.

Genetic patterns

To investigate the genetic underpinnings of simulated reaction norms, we defined replicate simulations as either TSD or GSD, determined by whether or not GLMs showed a significant ($P < 0.000125$) relationship between yearly temperature and cohort sex ratios across the last 20-years of each replicate simulation. Of our 400 replicate simulations across climatic parameter space defined by long-term mean and variation of annual mean temperature during gestation, 138 showed a significant effect of yearly temperature (T_y) on offspring sex over the last 20 cohorts and were, therefore, classed as TSD whilst 262 were classed as GSD (Figure 7). Simulation replicates resulting in GSD were found throughout the parameter space, whilst TSD simulation replicates were found where interannual variation in temperatures (SD_T) was low, except when long-term mean temperatures (T_m) were above 17 ^{o}C, when

TSD replicates also occurred at where interannual variation in temperatures (SD_T) was high.

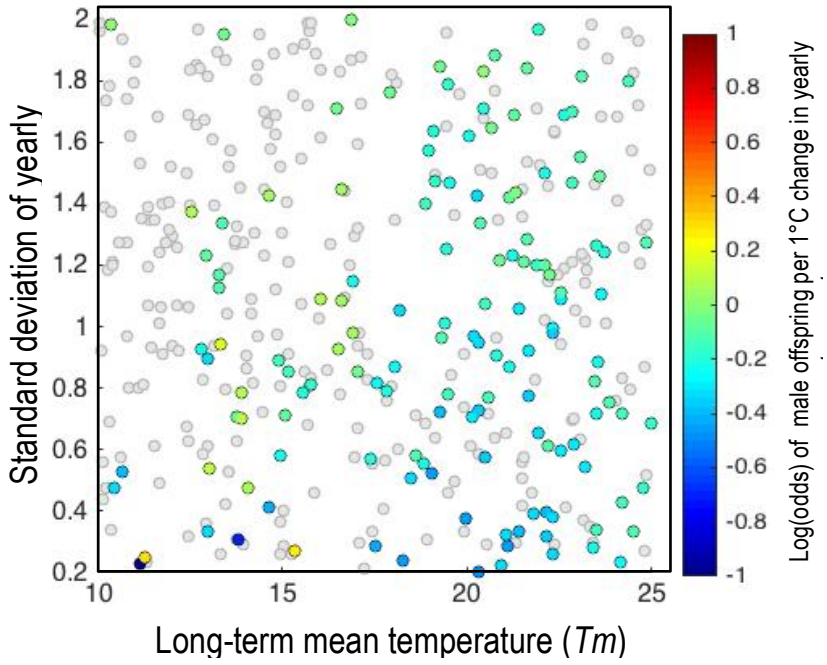

Figure 7: Results of 400 replicate simulations across climatic parameter space defined by a long-term mean of mean (T_m) and standard deviation (SD_T) of maximum temperatures during the gestation period (T_y). Grey circles indicate replicate simulations with no significant relationship (GSD) between temperature (Ty) and the cohort sex ratio over the last 20-years of replicate simulations (P < 0.000125). Filled circles represent replicate simulations with a significant relationship between yearly temperature and the cohort sex ratio over this period. The colour of filled circles indicates the strength of the effect (a change in the log(odds) ratio of males: females) for each 1 ^oC change in yearly temperature. A factor change > 0 indicates male skewed cohorts at high temperatures and < 0 indicates female skewed cohorts at high temperatures.

Among TSD replicate simulations (those with a significant relationship between temperature and offspring sex), 111 lost one sex chromosome allele through the course of the simulation (i.e., no males or females were heterozygous for sex chromosome alleles, all were XX). In only one TSD replicate simulation were all males heterozygous for sex chromosome alleles (i.e., XY). In the remaining 26 TSD replicate simulations, the proportion of homozygous (i.e., XX) males varied between 0.01 and 0.60 (see Supplementary Figure S2). Thus, the majority of TSD replicate simulations resulted in 'true TSD' (an absence of sex chromosomes), but a few were

the result of sex reversal occurring at extreme temperatures. Sex reversal of this type could explain the relationship between temperature and sex ratios in our lowland population, where sex ratios are more highly skewed towards males in cool years than towards females in warm years. Indeed, genetic evidence suggests that in the lowland population sex is partially determined genetically (i.e., a mixed system with sex reversal; Hill *et al.*, 2018). Of the 262 GSD simulation replicates (i.e., those with no significant relationship between temperature and offspring sex), 252 retained both sex chromosome alleles. Of these, 98 had only heterozygous (XY) males and only homozygous (XX) females (i.e., no sex reversal) and in a further 151 more than 90% of males were heterozygous. Thus, an XY GSD system, with occasional sex reversal, was maintained in the majority of GSD replicate simulations. In the 10 GSD replicate simulations in which one of the sex chromosome alleles was lost, we noted that the values of the threshold allele (τ) bifurcated via disruptive selection (see Supplementary Figures S9 & S11) such that both τ alleles in females had similar high values, whilst males had one τ allele with a high value, and one with a low value. Effectively, this represents the co-option of the τ allele as a sex-determining locus. Thus, populations that lost a sex chromosome allele through chance were able to, nevertheless, evolve a GSD system.

Of the 138 TSD replicate simulations, the majority (122) showed a skew towards males at low temperature and towards females at high temperatures, consistent with patterns from our empirical work, and with theoretical expectations. Whilst 16 replicate simulations showed a skew towards males at high temperatures, these had a comparatively weak effect of temperature on offspring sex (Figure 7). Across TSD replicate simulations, the strength of the effect of yearly temperature during gestation on cohort sex ratios varied between a factor change in the odds ratio of 0.17 and 1.74 for each increase in T_y of 1 °C. TSD replicate simulations with a skew towards males at high temperatures were more commonly found when the long-term mean temperature (T_m) was low, whilst those with a skew towards females at high temperatures were distributed across all values of T_m (Figure 7). Among TSD replicate simulations with a skew towards females at high temperatures, the strongest effects of a 1 °C change in yearly temperature were found across long-term mean yearly temperatures (T_m) and decreased as the variation in temperatures (SD_T) rose (Figure 7).

Climate, past and present

All five climate projection models showed substantial agreement in patterns of long-term mean maximum daily temperature during the gestation period (October 1st and December 31st; T_m) for both the present (1996-2016) and future (2080-2100) time periods. These models projected a rise in mean temperatures in all geographical areas (Supplementary Figure S7). Patterns of the interannual variability in temperatures during gestation between years (SD_T), however, varied among models for both periods (Supplementary Figure S7). Despite this difference, predictions of the shape of reaction norms in populations across the landscape were broadly consistent amongst climate models for both present (1996-2016) and projected (2080-2100) climates. We, therefore, show results from derived from the mean values of the five climate arrays (Figure 8) and present the results from individual climate projections as supplementary information (Supplementary Figures S7 & S8).

Present-day climates across Tasmania during the gestation period were characterised by warm mean temperatures in north-eastern and eastern coastal areas, with especially high temperatures in inland eastern areas. Western and north-western coastal areas had intermediate mean temperatures and central highland areas had very low mean temperatures (Figure 8A). In general, interannual variation in these temperatures was low (<0.5 SD) in all areas, peaking in south-western coastal and inland eastern areas (Figure 8B). Projected climates (2080-2100) showed an increase of mean temperature during the gestation period across all areas (Figure 8C) and a rise in variation of these temperatures in all areas other than the north-west, northern coast and southern coast. Variation was especially high in inland eastern areas (Figure 8D).

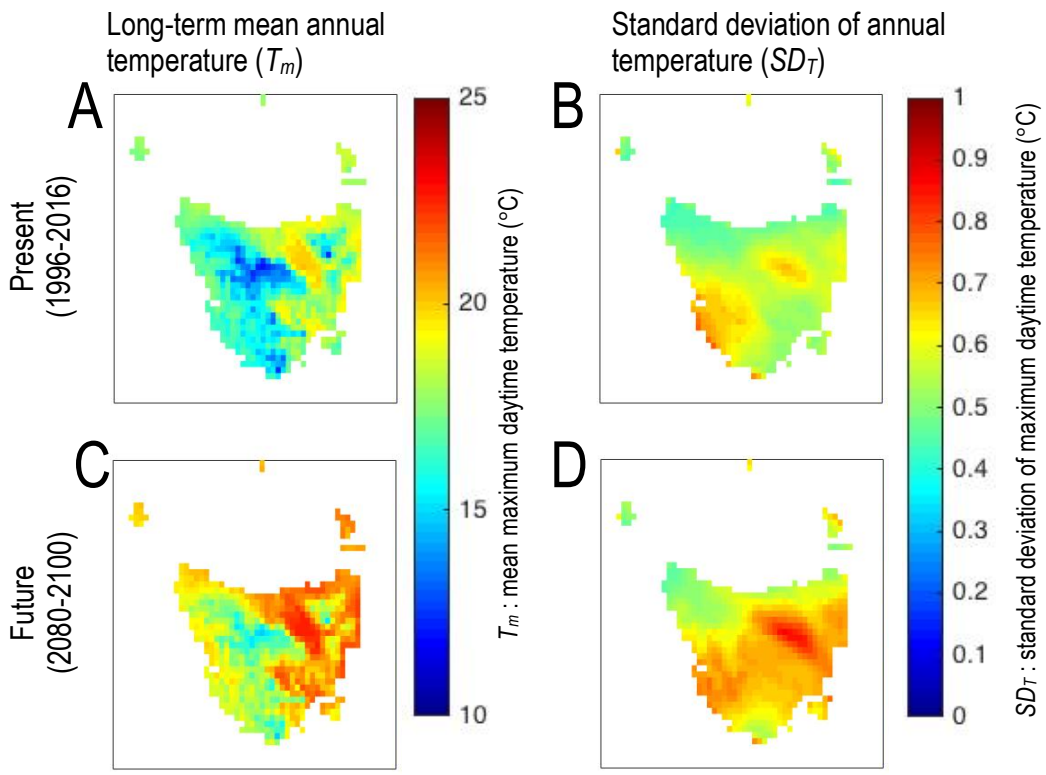

Figure 8: Mean of five climate projection results of the present (1996-2016) and future (2080-2100) 20-year mean (T_m) and standard deviation (SD_T) of annual mean maximum daytime temperature over the gestation period (Oct 1st – Dec 31st).

Selection across landscapes

Across the landscape, under present (1996-2016) climatic conditions (Figure 9A), responses to a 1 °C change in temperature ranged between a factor change in the odds ratio of male to female offspring of a strong effect: 0.73 (log(odds) = -0.30) to a weak effect: 0.98 (log(odds) = -0.302) with a narrow transitional zone between areas predicted to evolve strong and weak effects. We found a strong effect of temperature on cohort sex ratios in areas with relatively high temperatures and low variation in temperatures (northern and eastern coastal areas). We found the weakest effect of temperature on offspring sex in central highland areas with low temperatures and intermediate variation in temperatures (Figure 9A). At our long-term study sites the strength of the effect of temperature on cohort sex ratios was a factor change in the odds ratio of male to female offspring of 0.76 (log(odds) = -0.27) and 0.96 (log(odds) = -0.04) for each 1 °C change in temperature for the lowland and highland sites,

respectively (Figure 5). When these were scaled by the interannual standard deviation in temperatures, these translated to expected sex ratio skews in the lowland population of 0.41 in warm years (+2 SD) and 0.59 in cool years (-2 SD) and in the highland population of 0.48 in warm years and 0.52 in cool years. These patterns conformed to patterns found in our long-term data (Cunningham *et al.* 2017), to our expectations from the conceptual model and to findings from Pen *et al.* (2010).

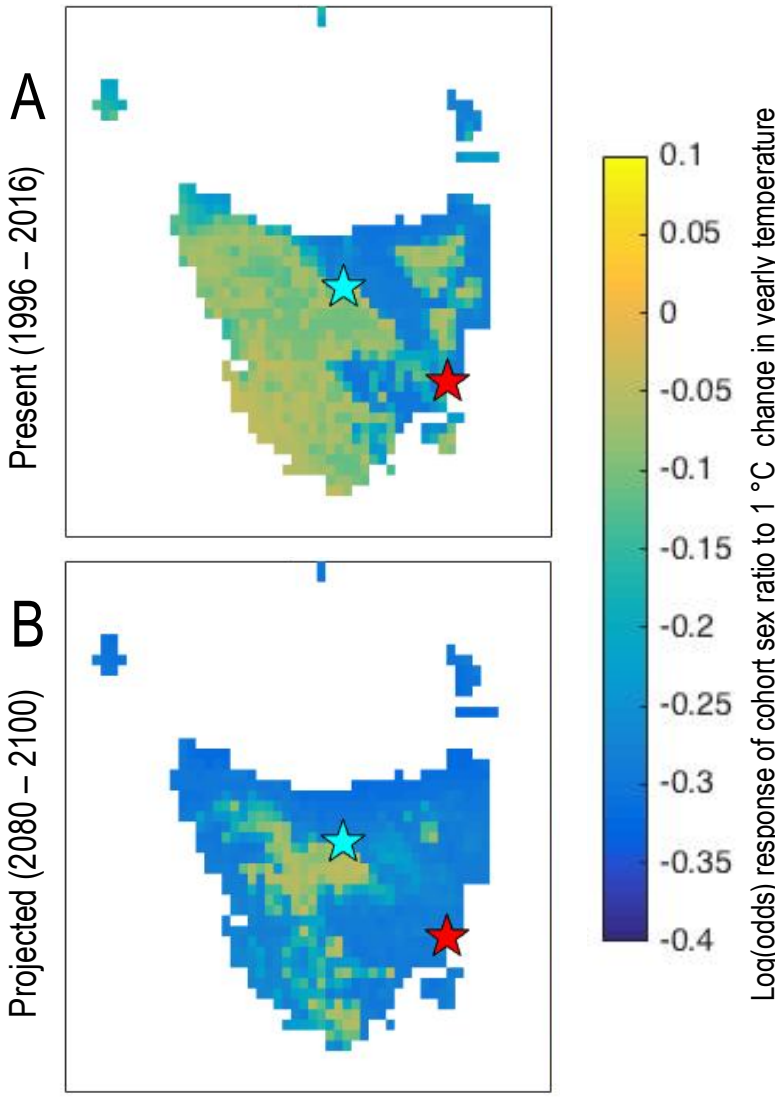

Figure 9: Modelled strength of the direction of selection for the effect of a 1 °C change in yearly temperature on a change in the log(odds) ratio of males: females across the present (1996-2016) and projected (2080-2100) Tasmanian climatic landscape from thin-plate spline interpolation model fit to the mean of five climate projection models. Stars show locations of our highland (blue) and lowland (red) long-term sites. Values above 0 indicate a skew towards males at high temperatures, whilst those below 0 indicate a skew towards females at high temperatures.

Under projected (2080-2100) climates, selection for reaction norms remained approximately the same as under current climatic conditions in areas predicted to evolve a strong effect of temperature on offspring sex (i.e., TSD) under current climatic conditions. Across the landscape selection favoured a weak response of sex to temperature (i.e., GSD) in only a few locations characterised by very low mean temperatures. In all other areas selection under altered climatic conditions favoured a strong response (i.e., TSD; Figure 9B).

Sex ratios in a warming climate

To predict how sex ratios vary across the landscape within the range of temperatures experienced, we scaled the response of offspring sex to a 1 °C change in temperature by the standard deviation of these temperatures between years (SD_T; see *Methods*, above). We found that, under present day climatic conditions (1996-2016; Figure 10A), in populations predicted to evolve a strong effect of temperature on sex (i.e., TSD; Figure 9A) sex ratios (the proportion of male offspring) would vary between 0.39 (i.e., a skew towards females) in warm years (+2 SD) and 0.61 (i.e., a skew towards males) in cool years (-2 SD). In locations predicted to evolve a weak effect of temperature on offspring sex, sex ratios would vary little among years.

When the pattern of predicted reaction norms of sex determination across the landscape was applied to climate projections for the period 2080-2100 (Figure 10B), we found that sex ratios in populations in which a 1 °C change in temperature was predicted to have a weak effect on sex ratios (i.e., GSD) will vary little between years. Across the landscape sex ratios in cool years (-2 SD) will be similar to those observed under current climatic conditions in warm years. In populations with a strong effect of a 1 °C change in temperature on offspring sex (i.e., TSD), sex ratios will be skewed towards females in all years. In years of mean (2080-2100) temperature, cohort sex ratios in these populations will have a sex ratio of 0.28 and will vary between 0.21 in warm years (+2 SD) and 0.39 in cool years (-2 SD).

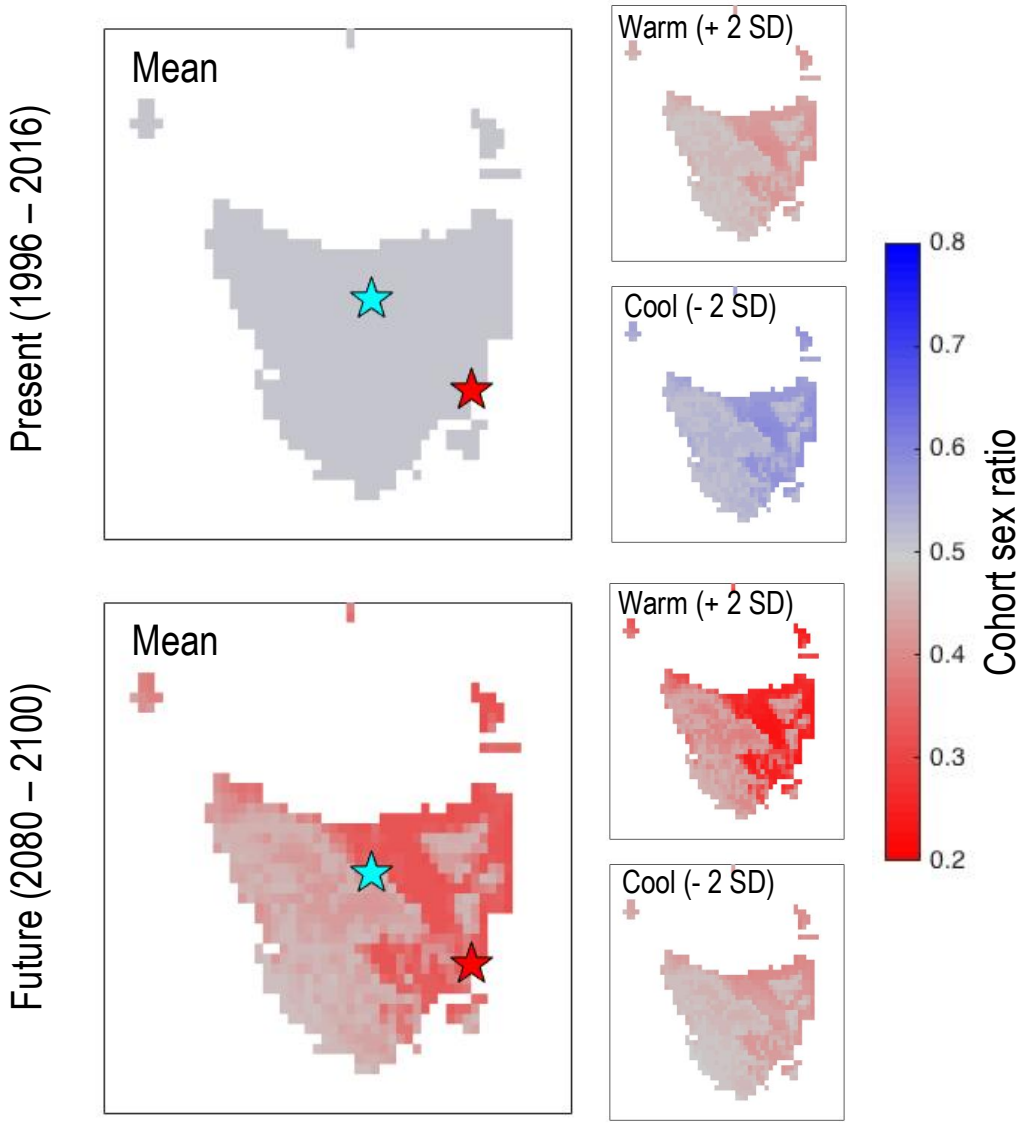

Figure 10: Predicted sex ratios (proportion males) in populations of *Niveoscincus ocellatus* across the landscape of Tasmania in years with mean, warm (+2 SD) and cold (-2 SD) temperatures under present (1996-2016) and future (2080-2100) climatic conditions.

Discussion

Within species, populations are likely to be adapted to local climatic conditions. How selective pressures shape the patterns of phenotypically plastic traits across climatic landscapes, however, is often difficult to determine in practice. From the results of an individual-based evolutionary simulation model, we predicted how selection would shape thermal reaction norms of sex determination across a climatic parameter space. Using these models, coupled with downscaled climate projections, we predicted the thermal reaction norms of sex determination across present day climatic landscapes. Applying these models to projections of future climates, we predicted how patterns of selection for the shape of reaction norms will be altered over the coming century and how sex ratios of offspring within populations will be affected by changing climates.

Selection across parameter space

Across climatic parameter space defined by mean and standard deviation of annual temperatures during the gestation period of *Niveoscincus ocellatus*, we found that both the long-term mean and variation in annual temperatures over the gestation period affected how selection would shape the strength of the relationship between yearly temperature and offspring sex ratios (i.e., reaction norms of sex determination). Specifically, we found that selection favouring a strong effect of temperature on offspring sex (i.e., TSD) peaked at intermediate long-term mean temperatures, declining at both low and high long-term mean temperatures and as interannual variation in temperatures increased. These patterns are driven by the relative fitness of early and late born females peaking at intermediate temperatures. Indeed, the relative fitness effect of date of birth declines at low mean temperatures because, irrespective of dates of birth (which take place over a short period), few females mature early. Similarly, the relative fitness of late- and early-born females declines at high mean temperatures because, despite births taking place over a longer period, all females have a high probability of maturing early, irrespective of their date of birth.

There was no position in climatic parameter space defined by the long-term mean and interannual variation in temperature, where sex ratios would be skewed to the extent that single sex cohorts would be produced within the range of temperatures

likely to be experienced. This comparatively shallow relationship conforms to patterns we have observed in *N. ocellatus*, but differs from 'switch-like' patterns in many TSD species (e.g., Janzen & Paukstis, 1991; Congdon *et al.* 1995; Inamdar *et al.*, 2012; Gomez-Saldarriaga *et al.*, 2016; Marco *et al.*, 2017). An explanation for this shallow reaction norm is that, because *N. ocellatus* is short-lived in comparison to many other TSD species (e.g., crocodiles and turtles), too steep a reaction norm would result in highly skewed sex ratios in the breeding population, counteracting the benefits of early maturation. Where TSD population's reaction norms are shallow, as in *N. ocellatus*, identifying TSD is more difficult than in species with switch-like reaction norms.

Importantly, we found that simulation replicates resulting in no significant effect of temperature (i.e., GSD replicates) occurred throughout climatic parameter space. In these simulation replicates, neither gene of main effect was lost and GSD was, therefore, maintained. Thus the probability of transitions from GSD to TSD rose at intermediate temperatures, but there was no position in parameter space where this transition was assured within the simulated time. Interestingly, we found that transitions are not only possible from GSD to TSD (via the loss of a gene of major affect) but also from TSD to GSD via the bifurcating of values of the threshold (τ) alleles. This result confirms results from Pen *et al.* (2010), in which simulations parameterised with data from our long-term highland (GSD) population, similarly evolved a 'novel genetic element' of major effect in those cases where a gene of major effect was lost through chance. In both studies, threshold alleles were co-opted as novel sex determining genes, leading to the evolution of GSD without genes of major effect (see Supplementary Figure S11). This suggests a possible mechanism by which species with TSD may evolve novel sex chromosomes, i.e., GSD (see Graves, 2013; Janes *et al.*, 2014; Ezaz *et al.*, 2017; Montiel *et al.* 2017) and a simplification of the Quinn *et al.* (2007) model (see *Methods*), such that sex determination may be modelled without the need for genes of major effect, corresponding to alleles on sex chromosomes.

These results show that, in species occupying a broad climatic range, populations may differ in sex determination system, dependent on the extent to which environmental variables have sex-specific relative fitness consequences.

Consequently, patterns of sex determination can alternate along environmental gradients if the greatest relative fitness difference occurs in intermediate environments. This pattern, where TSD occurs at intermediate climates, is similar to that reported in fish of the *Menidia* genus. In these fish, TSD occurs at mid latitudes and GSD at both high and low latitudes (Conover & Heins, 1987; Yamahira & Conover, 2003). Similarly, in the bearded dragon *Pogona vitticeps*, sex reversal has been found in populations located at intermediate latitudes, but not in populations further north or south (Holleley *et al.* 2015). In these taxa, populations with TSD or sex reversal, thus occupy a narrow climatic window in which associating offspring sex with temperature is an advantage. In *N. ocellatus*, shifts in climate, either a change in mean temperatures (warmer or colder) or an increase in interannual variation, if they are of sufficient magnitude, may result in populations with TSD transitioning to GSD and vice versa. Historically, climatic fluctuations have occurred in both these directions (Goede, 1994; Xia, *et al.*, 2001; Petherick *et al.*, 2011). Thus, in species where temperature or other environmental variables have similar sex-specific relative fitness effects, especially in taxa known to be labile for sex determination system, transitions between sex determination systems may oscillate over relatively short timescales (see Quinn *et al.*, 2011; Holleley *et al.*, 2015; Holleley *et al.*, 2016).

Selection across landscapes

When applied to the present-day climatic landscape of Tasmania, our model translated to a distribution of the shape of reaction norms consistent with empirical data. Populations in which selection favoured a weak effect of yearly temperature on offspring sex (GSD) were found in regions with low mean temperatures and high interannual variation in temperatures, whereas in warm, stable areas, selection favoured a comparatively strong effect of temperature on offspring sex (TSD). Indeed, we found that, across the landscape, reaction norms of sex determination were somewhat binary, resulting in either strong (i.e., TSD), or weak (i.e., GSD) responses of offspring sex to temperature, and that transition between these two extremes occurred over a comparatively narrow geographic space.

Interestingly, these results were consistent among all five climate models, which projected similar long-term mean temperatures but very different patterns of interannual variation in temperature. This suggests that patterns of alternative sex determination in *N. ocellatus* across the climatic landscape can be explained, primarily, by the selective effect of long-term mean temperatures, rather than of interannual variation in these temperatures. Reaction norms with a strong effect of temperature on sex were favoured in warm, highly variable regions because the long-term mean of annual temperatures during gestation in these regions corresponds to the position in climatic parameter space where selection for the evolution of TSD peaks under present climatic conditions. Whilst variability in these temperatures is high, it is not sufficient to overwhelm the selective advantage of TSD. Nevertheless, the patterns across climatic parameter space that we found, demonstrate that climatic variability may be important, especially as long-term mean temperatures diverge from the selective peak at 21°C. A change in climate, either towards colder or warmer long-term means, or increasing variation, therefore, will tend to shift these populations away from the selective peak of TSD.

When we applied our model to future (2080-2100) climatic landscapes we found that the selective pressure for TSD will remain high across regions predicted to have TSD in present-day climatic conditions. Indeed, the distribution of populations with selection for a weak, or no effect of temperature on offspring sex (i.e., GSD), will contract to the coldest and most variable climatic regions. Thus, we can expect the strength of selection for TSD to become comparatively homogeneous across the environment by 2100. If climates remain stable at these temperatures, TSD may become ubiquitous across *N. ocellatus* populations. It is likely, however, that both interannual variability and mean temperatures will continue to rise beyond 2100, especially if net greenhouse gas emissions continue past 2100 (Collins *et al.*, 2013). Should climates continue to warm beyond 2100 and variability in these temperatures continues to increase we might see a transition in observed patterns. As mean temperatures in lowland regions rise above the peak of selective pressure for TSD, and as these temperatures become more variable, the relative selective pressure for GSD exerted by temperature variability may result in selection for GSD in populations that currently have TSD. Thus, if climatic patterns are sufficiently changed, highland populations may transition from GSD to TSD, and lowland

populations from TSD to GSD. Furthermore, during the last glacial maximum, populations that currently have TSD may have experienced environmental conditions favouring GSD. Indeed, our highland (GSD) site appears to have been recolonised from lowland areas following the last glacial maximum (Cliff *et al*., 2015) and genetic evidence suggests that sex is partially determined genetically (i.e., a mixed system with sex reversal) in the lowland population (Hill *et al.*, 2018). GSD may, therefore, represent the ancestral state of both highland and lowland populations. Thus, in lowland populations TSD may be a transitive condition between episodes of GSD. Patterns of sex determination may constantly fluctuate across the range of species with intraspecific divergence in sex determination (e.g., Conover & Heins, 1987; Holleley *et al.* 2015) over evolutionary time.

Sex ratios in a warming climate

The predicted magnitude of cohort sex ratio skews varied between time periods. Under present day climatic conditions, we found that sex ratios in populations with TSD (i.e., a strong effect of temperature on offspring sex) would vary between 0.39 (a female skew) in warm years and 0.61 (a male skew) in cold years. In cold, variable regions, where selection favours GSD, sex ratios will remain balanced. Observed sex ratio skews at our lowland study site (Wapstra *et al.*, 2009; Pen *et al.*, 2010; Chapter 2: Cunningham *et al*., 2017; Chapter 3: Gruber *et al.* 2018) are consistent with those predicted from our model. As climates warm towards the end of the century, sex ratios in populations with TSD will become increasingly female-skewed in all years. Indeed, in 2100 sex ratios in cold years will be similar to those experienced in warm years in 2016. These more extreme female biases, however, will not result in single sex cohorts, even in extreme years. Whilst biased sex ratios can affect population persistence (Ginsberg & Milnergulland, 1994; Wedekind, 2002; Rankin & Kokko, 2007; Jenouvrier *et al.*, 2010). In species in which males mate with multiple females, such as *N. ocellatus*, female skewed ratios are unlikely to lead to population collapse, unless the skew in the breeding population is extreme enough that some females fail to mate (Rankin & Kokko, 2007). Thus, as we have previously argued (see Chapter 2: Cunningham *et al.*, 2017), TSD populations of *N. ocellatus* are unlikely to be at high risk from warming climates, because offspring sex ratios, even in especially warm

years are unlikely to exceed a ratio of 1 male to 4 females. Therefore, population collapse due to female skewed sex ratios is unlikely in this species.

Summary

Our results demonstrate that, among taxa known to be labile for sex determination system, interpopulation divergence may be more widespread than is realised, even across relatively constrained geographic areas, especially in species occupying a wide climatic range. Thus, researchers should be cautious of defining such species as uniform in sex determination system if results are derived from single populations, especially in taxa known to have lability in sex determining systems such as many lizards and fish (e.g., Conover, 1984; Ezaz *et al.*, 2009; Holleley *et al.* 2014), or in which sex reversal can occur (e.g., Holleley *et al*., 2014; 2016). Indeed, our results demonstrate that under some scenarios, sex determination systems may alternate along environmental gradients. As climates warm and become more variable, selective pressures will be altered and current patterns of sex determination within populations may not remain optimal. Thus, in species currently considered to have solely TSD or GSD, some populations may transition between sex determination systems as climates change and selective pressures are altered.

Acknowledgements

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Ethics

All guidelines and procedures for the use of animals were approved by the University of Tasmania Animal Ethics Committee (A0017006, A0016736).

References:

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2014). *lme4: Linear mixed-effects models using Eigen and S4*. R package version 1.1-7

Bull, J.J. (1983). *The evolution of sex determination systems*. Menlo Park (CA): Benjamin/Cummings Publishing Company.

Bulmer, M.G. & Bull, J.J. (1982). Models of polygenic sex determination and sex ratio control. *Evolution*, **36**: 13-26.

Bull, J.J. & Bulmer, M.G. (1989). Longevity enhances selection of environmental sex determination. *Heredity*, **63**: 315-320.

Boyle, M., Hone, J., Schwanz, L.E. & Georges, A. (2014). Under what conditions do climate-driven sex ratios enhance versus diminish population persistence? *Ecology and Evolution*, **4**: 4522-4533.

Cadby, C.D., Jones, S.M. & Wapstra, E. (2014). Geographical differences in maternal basking behaviour and offspring growth rate in a climatically widespread viviparous reptile. *Journal of Experimental Biology*, **217**: 1175-1179

Charnov, E.L. & Bull, J. (1977). When is sex environmentally determined? *Nature*, **266**: 828–830.

Cliff, H.B., Wapstra, E. & Burridge, C.P. (2015). Persistence and dispersal in a Southern Hemisphere glaciated landscape: the phylogeography of the spotted snow skink (*Niveoscincus ocellatus*) in Tasmania. *BMC Evolutionary Biology*, **15**, 121.

Collins, M., Knutti, R., Arblaster, J., Dufresne, J.-L., Fichefet, T., Friedlingstein, P., Gao, X., Gutowski, W.J., Johns, T., Krinner, G. *et al.* (2013). Long-term Climate Change: Projections, Commitments and Irreversibility. In: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V. & Midgley, P.M. (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Congdon, J.D., Fischer, R.U. & Gatten, R.E. (1995). Effects of incubation temperatures on characteristics of American alligators. *Herpetologica*, **51**: 497-504.

Conover, D.O. (1984). Adaptive significance of temperature-dependent sex determination in a fish. *American Naturalist***, 123**: 297-313.

Conover, D.O. & Heins, S.W. (1987). Adaptive variation in environmental and genetic sex determination in a fish. *Nature*, **326**: 496-498.

Cunningham, G.D., While, G.M. & Wapstra, E. (2017). Climate and sex ratio variation in a viviparous lizard. *Biology Letters*, **13**: 20170218.

Cunningham, G.D., Fitzpatrick, L.J., While, G.M. & Wapstra, E. (2018). Plastic rates of development and the effects of thermal extremes on offspring fitness in a viviparous lizard. *Journal of Experimental Zoology*, **329:** 262-270.

Ezaz, T., Quinn, A.E., Sarre, S.D., O'Meally, D., Georges, A. & Graves, J.A.M. (2009). *Chromosome Research*, **17**: 91-98.

Ezaz, T., Srikulanth, K. & Graves, J.A.M. (2017). Origin of amniote sex chromosomes: an ancestral super-sex chromosome, or common requirements? *Journal of Heredity*, **108**: 94-105.

Ginsberg, J.R. & Milnergulland, E.J. (1994). Sex-biased harvesting and population-dynamics in ungulates – implications for conservation and sustainable use. *Conservation Biology*, **8**: 157-166.

Goede, A. (1994). Continuous early last glacial paleoenvironmental record from a Tasmanian speleotherm based on stable-isotope and minor element variations. *Quaternary Science Reviews*, **13**: 283-291.

Gomez-Saldarriag, C., Valenzuela, N. & Ceballos, C.P. (2016). Effects of incubation temperature on sex determination in the endangered Magdalena River turtle, *Podocnemis lewyana*, **15**: 43-53.

Graves, J.A.M. (2013). How to evolve new vertebrate sex determining genes. *Developmental Dynamics*, **242**: 354-359.

Grose, M.R., Barnes-Keoghan, I., Corney, S.P., White, C.J., Holz, G.K., Bennett, J.B., Gaynor, S.M. & Bindoff, N.L. (2010). *Climate Futures for Tasmania: general climate impacts technical report*, Antarctic Climate & Ecosystems, Cooperative Research Centre, Hobart, Tasmania.

Gruber, J., Cunningham, G.D., While, G.M., Wapstra, E. (2018). Disentangling sex allocation in a viviparous reptile with temperature-dependent sex determination: a multifactorial approach. *Journal of Evolutionary Biology*, **31**: 267-276.

Hays, G.C., Mazaris, A.D., Schofield, G. & Laloe, J.O. (2017). Population viability at extreme sex-ratio skews produced by temperature-dependent sex determination. *Proceedings of the Royal Society B- Biological Sciences*, **284**: 20162576.

Hill, P.L., Burridge, C.P., Ezaz, T., Wapstra, E. (2018). Conservation of sex-linked markers among conspecific populations of a viviparous skink, *Niveoscincus ocellatus*, exhibiting genetic and temperature-dependent sex determination. *Genome Biology and Evolution*, **10**: 1079-1087.

Holleley, C.E., Sarre, S.D., O'Meally, D., Graves, J.A.M, Ezaz, T., Matsubara, K., Azad, B., Zhang, X.W. & Georges, A. (2015). Sex reversal triggers the rapid transition from genetic to temperature-dependent sex. *Nature*, **523**: 79-+.

Holleley, C.E., O'Meally, D., Sarre, S.D. & Georges, A. (2016). Sex reversal in reptiles: reproductive oddity or powerful driver of evolutionary change? *Sexual Development*, **10**: 279-287.

Inamdar, L.S., Vani, V. & Seshagiri, P.B. (2012). A tropical oviparous lizard, *Calotes veriscolor*, exhibiting a potentially novel FMFM pattern of temperature-dependent sex determination. *Journal of Experimental Zoology Part A-Ecological Genetics and Physiology*, **317A**: 32-46.

Janes, D.E., Organ, C.L., Stiglec, R. O'Meally, D., Sarre, S.D .,Georges, A., Graves, J.A.M., Valenzuela, N., Literman, R.A., Rutherford, K. *et al*. (2014). Molecular evolution of Dmrt1 accompanies change in sex determining mechanisms in reptilian. *Biology Letters*, **10**: 20140809.

Janzen, F.J. (1994). Climate change and temperature-dependent sex determination in reptiles. *Proceedings of the National Academy of Science*s, **91**: 7487-7490.

Janzen, F.J. & Paukstis, G.L. (1991) Environmental sex determination in reptiles – ecology, evolution, and experimental design. *Quarterly Review of Biology*, **66**: 149-179.

Jenouvrier, S, Caswell, H., Barbraud, C. & Weimerskirch, H. (2010). Mating behaviour, population growth, and the operational sex ratio: a periodic two-sex model approach. *American Naturalist*, **175**: 739-752.

Le Galliard, J.F., Fitze, P.S., Ferriere, R., & Clobert, J. (2005). Sex ratio bias, male aggression, and population collapse in lizards. *Proceedings of the National Academy of Sciences*, **102**: 18231-18236.

Marco, M.V.P, Leiva, P., Iungman, J.L., Simoncini, M.S. & Pina, C.I. (2017). New evidence characterising temperature-dependent sex determination in broad-snouted caiman, *Caiman latirostris*, **12**: 78-84.

Matlab 2015b (2015). The MathWorks, Inc., Natick, Massachusetts, United States.

Mitchell, N.J. & Janzen F.J. (2010). Temperature-dependent sex determination and contemporary climate change. *Sexual Development*, **4**: 129-140.

Mitchell, N.J., Allendorf, F.W., Keall, S.N., Daugherty, C.H. & Nelson, N.J. (2010). Demographic effects of temperature-dependent sex determination: will tuatara survive global warming? *Global Change Biology*, **16**: 60-72.

Montiel, E.E., Badenhorst, D., Tamplin, J., Burke, R.L. & Valenzuela, N. (2017). Discovery of the youngest sex chromosomes reveals first case of convergent co-option of ancestral autosomes in turtles. *Chromosoma*, **126**: 105-113.

Petherick, L.M., Moss, P.T. & McGowan, H.A. (2011). Climatic and environmental variability during the termination of the last glacial stage in coastal eastern Australia: a review. *Australian Journal of Earth Sciences*, **58**: 563-577.

Pen, I. Uller, T., Feldmeyer, B., Harts, A., While, G.M. & Wapstra, E. (2010). Climate-driven population divergence in sex-determining systems. *Nature*, **468**: 436-U262.

Quinn, A.E., Georges, A., Sarre, S.D., Guarino, F., Ezaz, T. & Graves, J.A.M. (2007). Temperature sex reversal implies sex gene dosage in a reptile. *Science*, **316**: 411-411.

Quinn, A.E., Sarre, S.D., Ezaz, T., Graves, J.A.M. & Georges, A. (2011). Evolutionary transitions between mechanisms of sex determination in vertebrates. *Biology Letters*, **7**: 443-448.

R Core Team (2014). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.

Rankin, D.J. & Kokko, H. (2007). Do males matter? The role of males in population dynamics. *Oikos*, **116**: 335-348.

Robertson, B., Rehage, J.S. & Sih, A. (2013). Evolutionary novelty and the emergence of evolutionary traps. *Trends in Ecology and Evolution*, **28**: 552-560.

Sarre. S.D., Georges, A. & Quinn, A. (2004). The ends of a continuum: genetic and temperature-dependent sex determination in reptiles. *Bioessays*, **26**: 639-645.

Schwanz, L.E., & Janzen, F.J. (2008). Climate change and temperature-dependent sex determination: can individual plasticity in nesting phenology prevent extreme sex ratios? *Physiological and Biochemical Zoology*, **6**: 826-834.

Schwanz, L.E., & Proulx, S.R. (2008). Mutual information reveals variation in temperature-dependent sex determination in response to environmental fluctuation, lifespan and selection. *Proceedings of the Royal Society B-Biological Sciences*, **275**: 2441-2448.

Schwanz, L.E., Spencer, R.J., Bowden, R.M. & Janzen, F.J. (2010a). Climate and predation dominate juvenile and adult recruitment in a turtle with temperature-dependent sex determination. *Ecology*, **91**: 3016-3026.

Schwanz, L.E., Janzen, F.J. & Proulx, S.R. (2010b). Sex allocation based on absolute and relative condition. *Evolution*, **64**: 1331-1345.

Schwanz, L.E., Ezaz, T., Gruber, B. & Georges, A. (2013). Novel evolutionary pathways of sex-determining mechanisms. *Evolutionary Biol*ogy, **26**: 2544-2557.

Schwanz, L.E., Cordero, G.A., Charnov, E.L. & Janzen, F.J. (2016). Sex-specific survival to maturity and the evolution of environmental sex determination. *Evolution*, **70**: 329-341.

Shine, R. (1999). Why is sex determined by nest temperature in many reptiles? *Trends in Ecology and Evolution*, **14**: 186–189.

The tree of sex consortium (2014). Tree of sex: a database of sexual systems. *Scientific Data*, 1:140015

Uller, T., While, G.M., Cadby, C.D., Harts, A., O'Connor, K., Pen, I. & Wapstra, E. (2011). Altitudinal divergence in maternal thermoregulatory behaviour may be driven by differences in selection on offspring survival in a viviparous lizard. *Evolution.* **65**: 2313-2324.

van Dooren, T.J.M & Leimar, O. (2003). The evolution of environmental and genetic sex determination in fluctuating environments. *Evolution*, **57**: 2667-2677.

Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C, Fromentin, J.M., Hoegh-Guldberg, O. & Bairlein, F. (2002). Ecological responses to recent climate change. *Nature*, **416**: 389-395.

Wapstra, E. & O'Reilly, J.M. (2001). Potential 'costs of reproduction' in a skink: Inter- and intrapopulational variation. *Austral Ecology*, **26**: 179-186.

Wapstra, E., Swain, R., Jones, S.M. & O'Reilly, J. (1999). Geographic and annual variation in reproductive cycles in the Tasmanian spotted snow skink, *Niveoscincus ocellatus* (Squamata: Scincidae). *Australian Journal of Zoology*, **47**: 539-550.

Wapstra, E., Swain, R. & O'Reilly, J.M. (2001). Geographic variation in age and size at maturity in a small viviparous skink. *Copeia*, **3**: 646-655.

Wapstra, E., Uller, T., Sinn, D.L., Olsson, M., Mazurek, K., Joss, J. & Shine, R. (2009). Climate effects on offspring sex ratio in a viviparous lizard. *Journal of Animal Ecology*, **78**, 84-90.

Wedekind, C. (2002). Manipulating sex ratios for conservation: short term risks and long-term benefits. *Animal Conservation*, **5**: 13-20.

Xia, Q.K., Zhao, J.X. & Collerson, K.D. (2001). Early-mid Holocene climatic variations in Tasmania, Australia: multi-proxy records in a stalagmite from Lynds cave. *Earth and Planetary Science Letters*, **194**: 177-187.

Yamahira, K. & Connover, D.O. (2003). Interpopulation variability in temperature-dependent sex determination of the tidewater silverside *Menidia peninsulae* (Pisces: Atherinidae). *Copeia*, **1**: 155-159.

Chapter 6: Supplementary Information

Table S1: Number of litters, individual offspring and cohort sex ratios in a highland and a lowland population of *N. ocellatus* across a 16-year field study (2000-2015).

Season	Highland			Lowland		
	Number of litters	Number of offspring	Cohort sex ratio	Number of litters	Number of offspring	Cohort sex ratio
2000/2001	88	373	0.54	91	196	0.45
2001/2002	114	438	0.55	99	222	0.59
2002/2003	83	294	0.56	90	205	0.56
2003/2004	102	351	0.49	104	211	0.66
2004/2005	99	318	0.48	93	220	0.59
2005/2006	96	317	0.53	110	283	0.45
2006/2007	85	261	0.54	91	221	0.49
2007/2008	73	242	0.58	71	171	0.50
2008/2009	59	204	0.56	92	233	0.53
2009/2010	43	160	0.53	98	242	0.53
2010/2011	45	163	0.51	104	253	0.53
2011/2012	48	176	0.52	92	227	0.49
2012/2013	52	187	0.50	87	190	0.50
2013/2014	71	276	0.51	79	200	0.60
2014/2015	71	269	0.51	72	178	0.49
2015/2016	62	237	0.48	50	127	0.55
Total	1191	4266	0.52	1423	3379	0.53

Table S2: Proportion of females with known dates that were first recaptured and gave birth at ages 2, 3 & 4 in a two populations of *N. ocellatus* over a 16-year long-term study.

Site	Age 2	Age 3	Age 4
Lowland	48 / 148 (32%)	72 / 100 (72%)	17 / 28 (61%)
Highland	12 / 187 (6%)	128 / 175 (73%)	37 / 47 (78%)

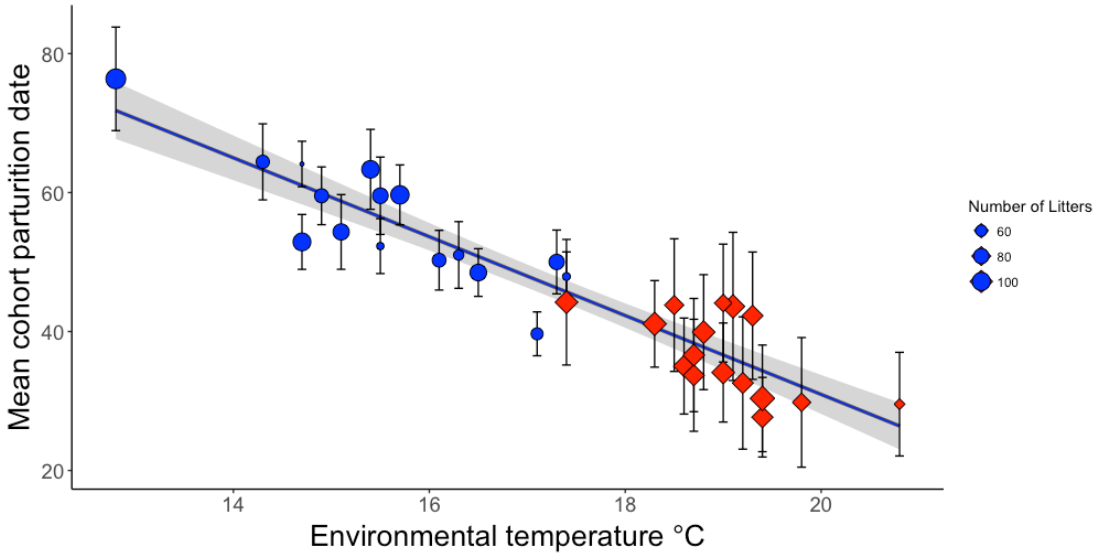

Figure S1: Relationship between yearly mean maximum temperature during the gestation period (Oct 1st – Dec 31st) and cohort mean date of birth across a 16-year long-term study of *Niveoscincus ocellatus* in a highland (blue) and lowland (red) population. Sizes of points indicate the number of litters produced in each year.

Table S3: Results of GLMs testing the effect of date of birth and site on the probability of immature females maturing at ages 2 and 3 in two populations (sites) of *N. ocellatus* (2000/2001 - 2011/2012).

	Site	Birth date	Site : Birthdate
Age 2	**χ^2_1 = 5.787; *P* = 0.016** **β = 1.190 ± 0.498 SE**	**χ^2_1 = 5.299; *P* = 0.021** **β = -0.037 ± 0.038 SE**	χ^2_1 = 0.015; *P* = 0.903 β = 0.005 ± 0.038 SE
Age 3	χ^2_1 = 0.009; *P* = 0.923 β = -0.045 ± 0.460 SE	χ^2_1 = 0.986; *P* = 0.321 β = -0.014 ± 0.014 SE	χ^2_1 = 1.938; *P* = 0.164 β = -0.044 ± -0.032 SE

Note: *P*-values, χ^2 statistics and β values for main effects are from reduced models excluding interactions. Significant effects are shown bold. β values for the effects here were used to parameterise the effects of date of birth (DOB_i) and long-term mean annual temperature (T_m) on the probability of females giving birth at age two in the simulation model assuming a linear relationship between T_m at the long-term study sites and the β coefficient effect of site.

Table S4: Coefficients used in the individual-based evolutionary simulation model. Coefficients derived from GLM and LM models (see text) fit to field data collected across a 16-year (2000-2015) field study of highland and lowland sites are noted below.

Variable	Equation
Long-term mean temperature	$T_m \sim U(10, 25)$
Standard deviation in temperatures between years	$SD_T \sim U(0.2, 2)$
Yearly temperature	$T_y \sim \mathcal{N}(T_m, SD_T)$
Cohort mean date of birth*	$DOB_m = -4.596 \times T_y + 123.204$
Standard deviation in birth dates within cohorts*	$SD_{DOB} = -6.8 + 0.8$
Litter date of birth	$DOB_i \sim \mathcal{N}(DOB_m, SD_{DOB})$
Effective development temperature*	$T_i = \frac{DOB_i - 123.204}{-4.596}$
Site effect of long-term mean temperature on odds of females maturing at age 2§	$M_s = -3.3151 + 0.2624 \times T_m$
Odds of females maturing at age 2§	$Odds_{mat} = \exp(-0.5 + (-0.0367 \times DOB_i + M_s))$
Probability of females maturing at age 2	$P_{mat} = \frac{Odds_{mat}}{1 + Odds_{mat}}$

Note: Parameter values derived from coefficient estimates from (*) linear models and (§) generalised linear models fit to long-term empirical data from a 16-year field study.

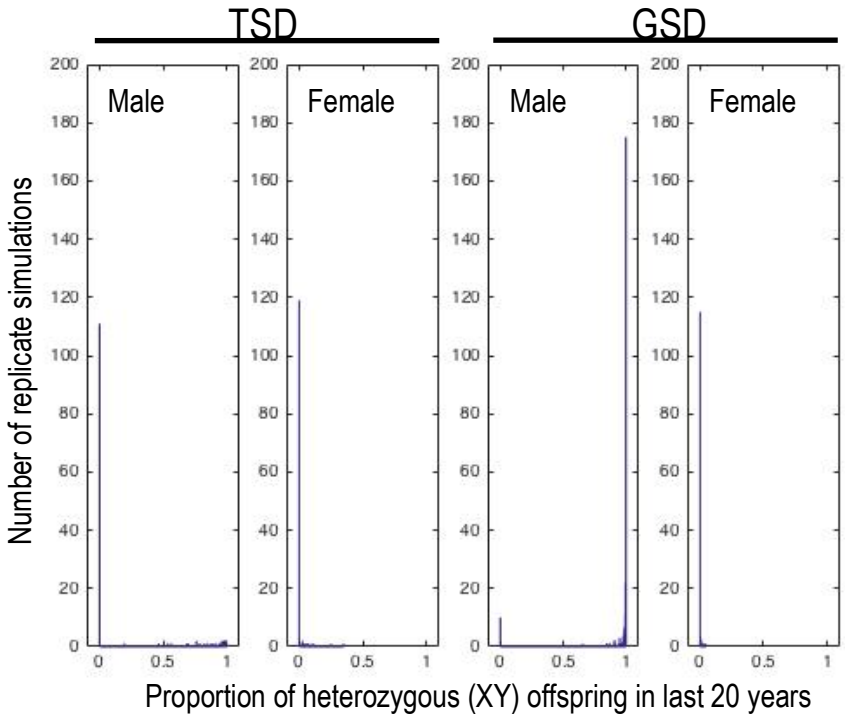

Figure S2: Histogram showing the number of male and female offspring heterozygous for genes of major effect ($\kappa_A \kappa_a$) across the final 20 years of replicate simulations resulting in the evolution of TSD and the maintenance of GSD.

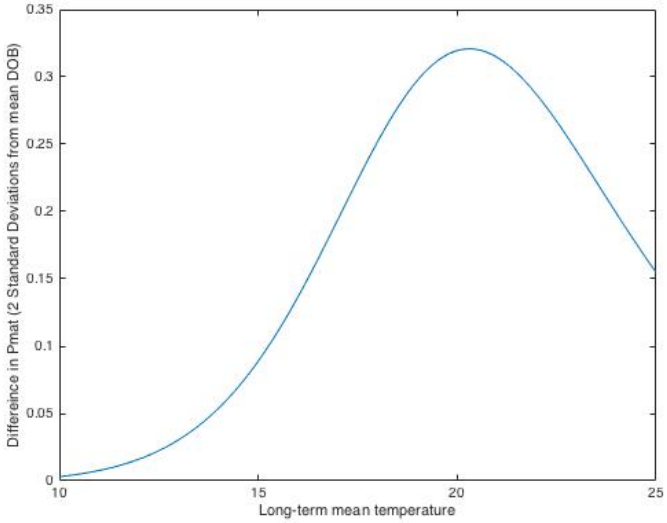

Figure S3: Difference in the probability of maturing at age two between females born +2 and -2 standard deviations from the mean date of birth in a year in which annual temperature (T_y) is the long-term mean annual temperature (T_m) in simulated *Niveoscincus ocellatus* populations.

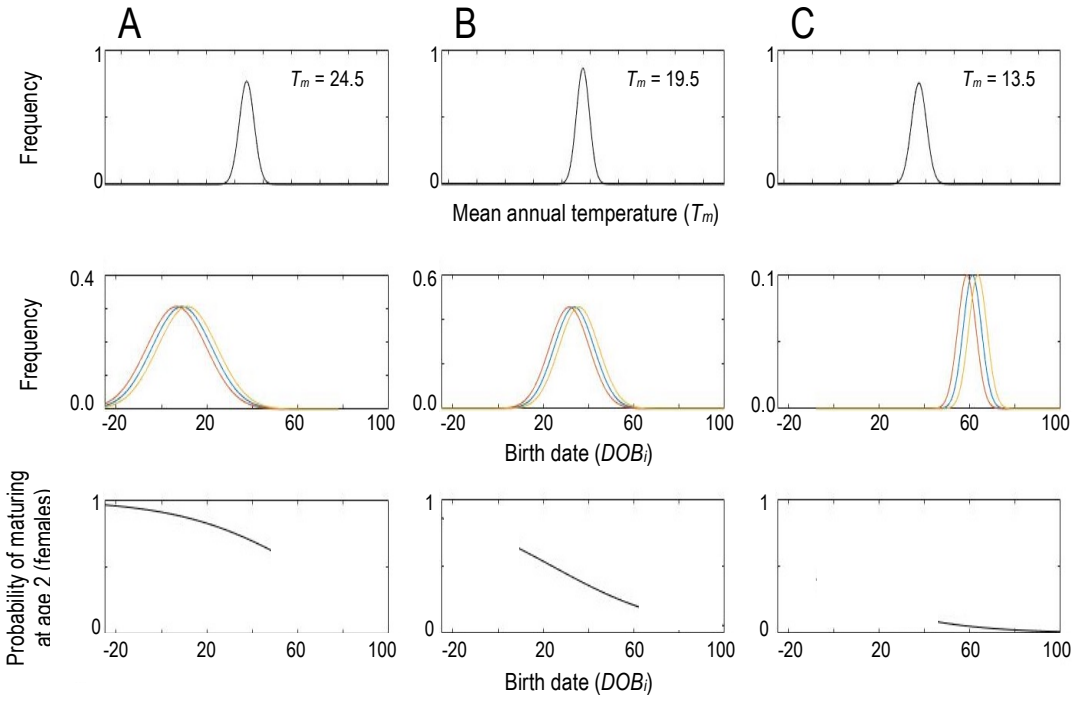

Figure S4: Effect of individual birth date on the probability of females maturing at age 2 in A) a hot scenario, B) a warm scenario & C) a cool scenario of simulated populations of *N. ocellatus.*

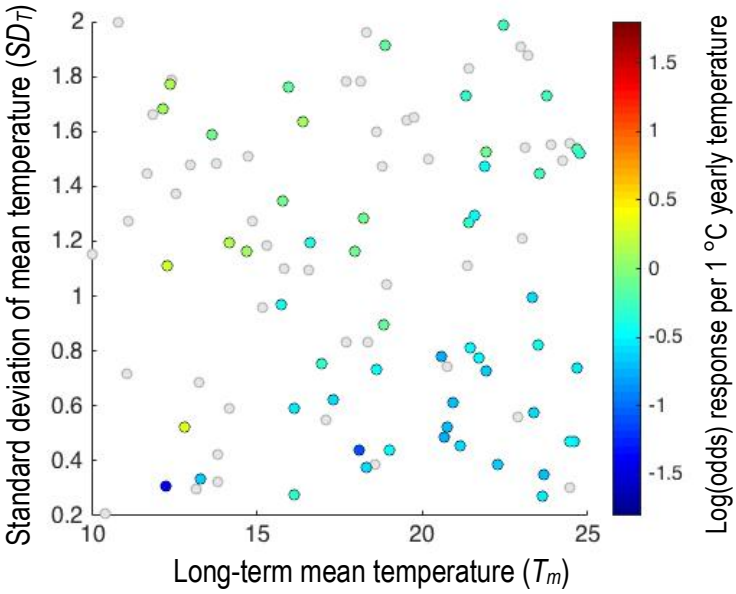

Figure S5: Results from 100 simulations run for 100,000 simulation-years showing the change in the log(odds) of male offspring per 1 °C (unscaled) change yearly temperature. Note, TSD was defined with at a significance of P < 0.0005 (i.e., a Bonferroni correction for 100 tests) as opposed to P < 0.000125 in the primary model.

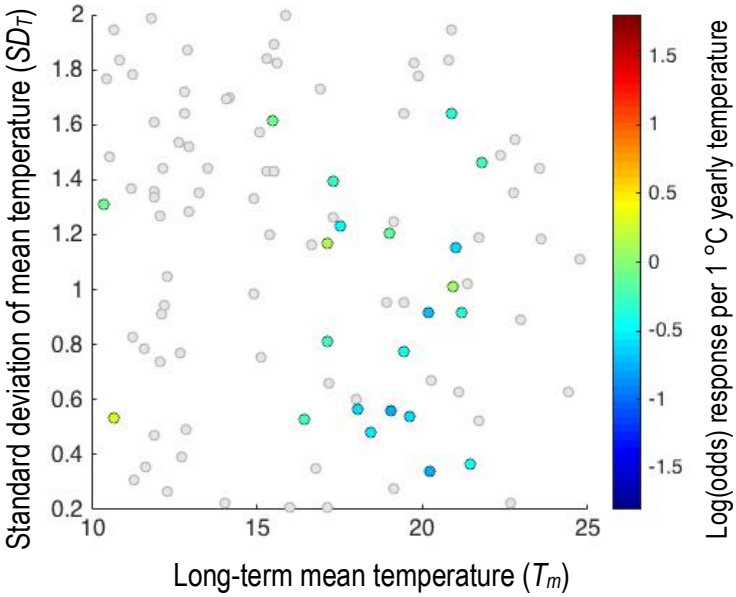

Figure S6: Results from 100 simulations run with alleles mutating with a probability of 0.05 (rather than 0.1 as in the primary model) showing the change in the log(odds) of male offspring per 1 °C (unscaled) change yearly temperature. Note, TSD was defined with at a significance of P < 0.0005 (i.e., a Bonferroni correction for 100 tests) as opposed to P < 0.000125 in the primary model.

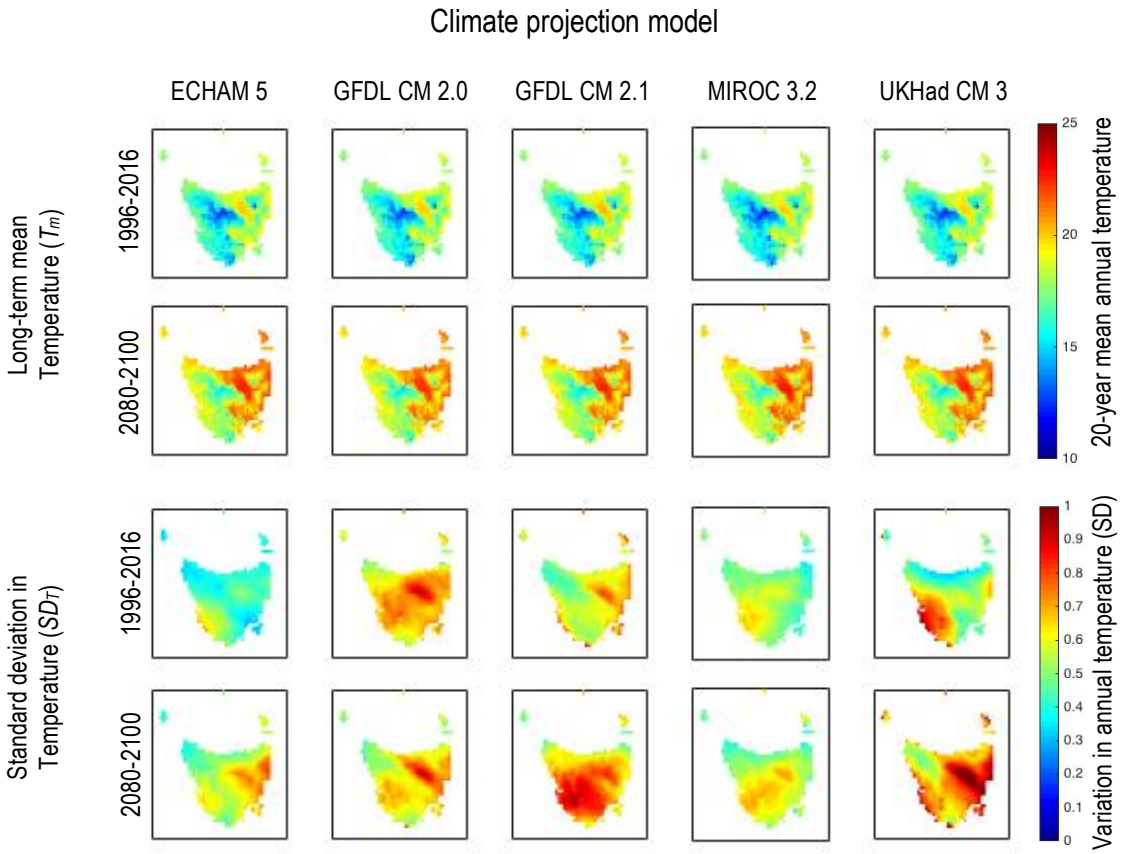

Figure S7: 20-year Mean and standard deviation of maximum daytime temperatures during the gestation period (Oct 1st – Dec 31st) according to five climate projection models in the present (1996-2015) and future (2080-2100).

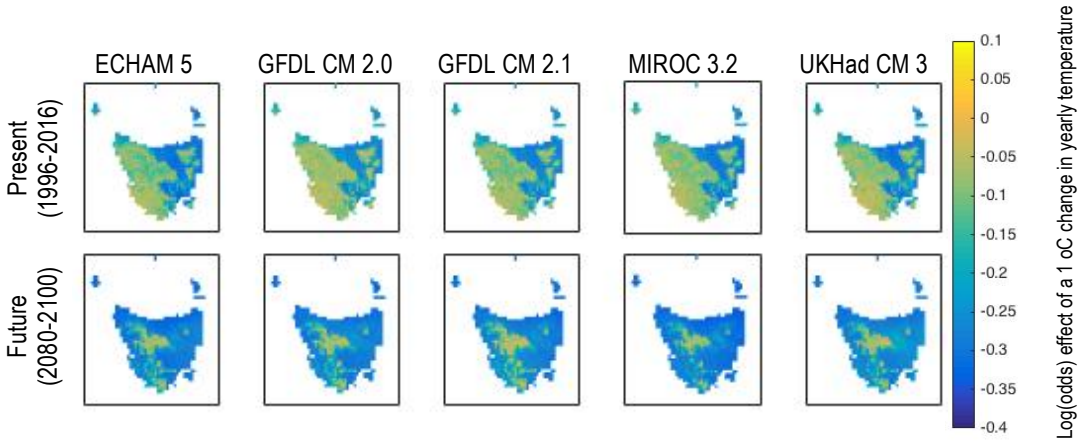

Figure S8: Modelled strength of the evolved effect of yearly temperature on cohort sex ratios in populations of *N. ocellatus* across the present (1996-2016) and future (1996-2016) climate of Tasmania.

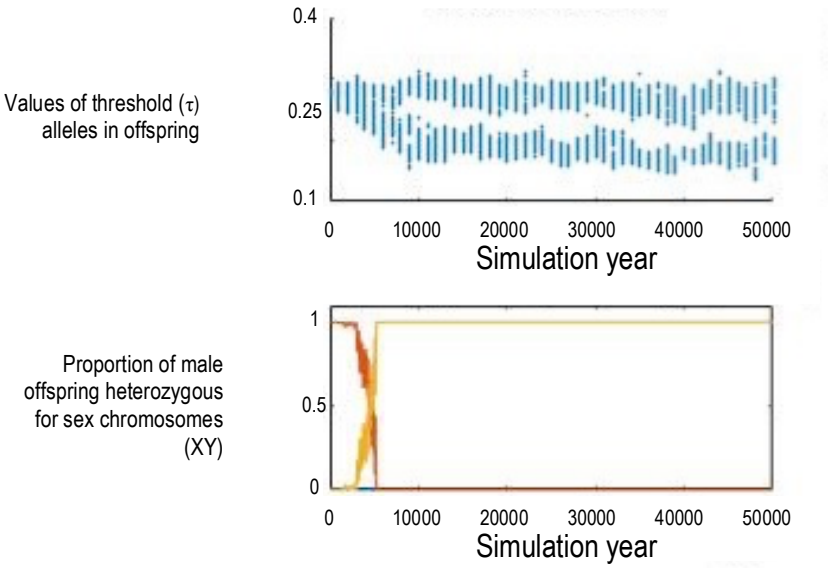

Figure S9: Evolution of a novel genetic element following the loss of an allele of main effect via the bifurcation of values of the τ allele.

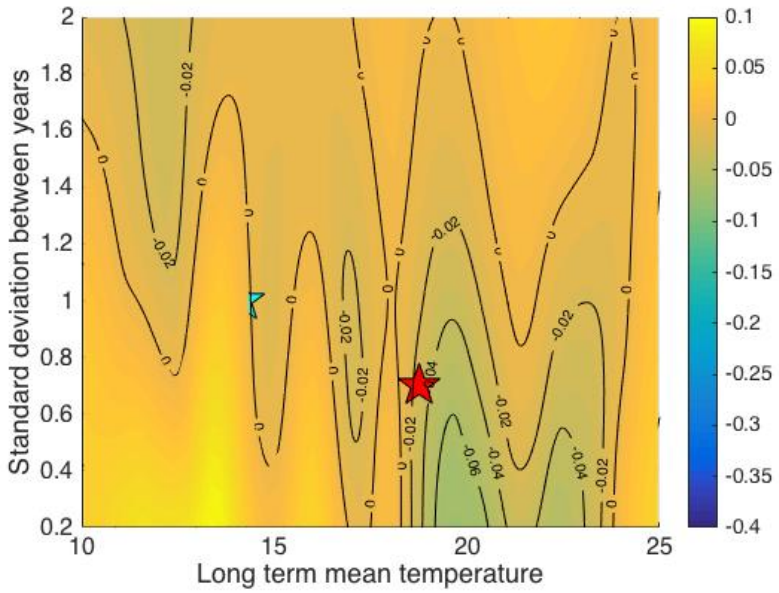

Figure S10: Thin plate spline interpolation of the relationship between yearly temperature (°C) and cohort sex ratio across climatic parameter space defined by mean (T_m) and standard deviation (SD_T) of annual mean temperature when the probability of females reaching maturity at age 2 is independent of date of birth. Values above 0 indicate a skew towards males at high temperatures, those below 0 a skew towards females at high temperatures. Stars indicate the position in parameter space of the lowland (red) and highland (blue) study sites.

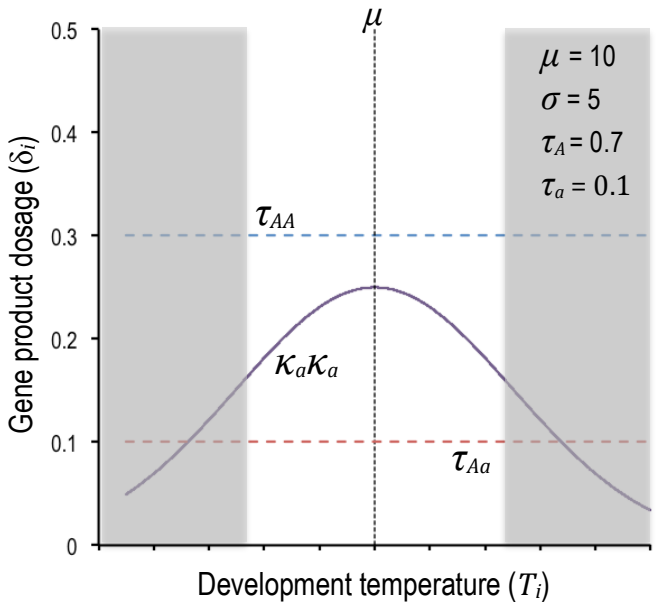

Figure S11: Sex determination via the evolution of a 'novel genetic element' due to bifurcation of allelic values of the threshold (τ) gene following the loss of a gene of major effect. All individuals are homozygous for κ. Individuals homozygous for the high value threshold allele develop as male. Individuals with heterozygous values for the threshold alleles develop as female.

Chapter 7:

General Discussion

Phenotypic plasticity describes how organismal traits change across environmental gradients and can be described at population, individual and genetic levels (Brommer, 2013). Whilst some forms of plasticity are non-adaptive consequences of environmental effects on organisms (e.g., due to physiological processes or limitations), plasticity can also be adaptive, allowing species to optimise their fitness in a range of environmental contexts (Ghalambor *et al.*, 2007). Thus, adaptive plasticity can be an important determinant of how populations will respond to altered climatic conditions (Nicotra *et al*., 2010; Lawson *et al.*, 2015; McLean *et al.*, 2016).

Key aspects of the environment that mediate plasticity, such as environmental means, variability and heterogeneity, can vary among locations. Because populations have evolved within specific ecological contexts (Hoffmann & Sgro, 2011; Beldade *et al.*, 2011), patterns of plasticity may vary within species, among populations, and among the individuals within them (Husby *et al.*, 2010; Valladares *et al.*, 2014). Thus, populations may differ in their responses to altered environmental conditions in the short-term via phenotypic plasticity. Over longer timeframes populations will either become extinct, or will adapt to changed conditions. The evolutionary response of a population may, however, itself be affected by patterns of plasticity. Phenotypic plasticity can either promote an evolutionary response by enabling population persistence in changed conditions, or can hinder evolution by supressing the effects of selection (Ghalambor *et al.*, 2007; Chevin & Lande, 2010; Chevin *et al.*, 2013; Kovach-Orr and Fussman, 2013). Populations are also likely to differ in how they respond to changes in environmental conditions on long-term evolutionary timescales because of differences among them in factors that affect genetic diversity, such as biogeographic history, demographics and connectivity. Understanding how plasticity evolves and the degree to which it varies among and within populations is of intrinsic biological interest because these patterns have significant evolutionary and ecological consequences (Fusco & Minelli, 2010; Valladares *et al.*, 2014). In the light of ongoing climate change, however, it has become increasingly important that we gain an understanding the adaptive significance of phenotypic plasticity and how patterns of plasticity vary among species, populations and individuals (Nicotra *et al.*, 2010; Valladares *et al.*, 2014). This is particularly true of traits that are plastic in response to climatic environmental factors, especially temperature (i.e., thermal plasticity).

Non-avian reptile species are excellent organisms in which to investigate thermal plasticity. This is because reptiles are ectotherms and, as such, much of their biology is heavily impacted by temperature. Consequently, thermal plasticity has been extensively studied in reptiles (e.g., Deeming, 2004; Noble *et al*., 2018; While *et al.*, 2018). Whilst plastic responses can occur at any stage during an organism's life, its effects during development are especially important (West-Eberhard, 2003). As a result, much of the research on thermal plasticity in reptiles has focused on the effects of temperature during development (i.e., thermal developmental plasticity). This research has shown that the thermal environment during early development can have substantial effects on a wide number of traits including incubation duration, developmental processes (e.g., gene expression), morphology, behaviour, performance, physiology, cognition and, in many species, sex. The phenotypic variation generated in response to the thermal environment has been shown to have fitness consequences in both the short-term (e.g., via increased post-hatching growth and survival; Andrews et al. 2009) and throughout life (e.g., future reproductive success; Warner & Shine 2008b).

The majority of work on the effects of the thermal environment in reptiles has focused on oviparous species. This is, perhaps, unsurprising. First because the majority of reptiles are oviparous and, secondly because, in these species, developmental temperatures are comparatively easy to manipulate via the use of incubators, giving researchers fine-scale experimental control over the developmental environment (see Noble *et al.*, 2018). In contrast, the developmental environment in viviparous species is much harder to manipulate. Whilst mothers in oviparous species can play some role in mediating the developmental temperature of their offspring (e.g., through laying date, nest depth and/or choice of nesting site), mothers in viviparous species are able to exert much greater control via basking behaviour (Morjan, 2003; Doody, *et al.*, 2006; Mitchell *et al.*, 2008; Schwanz & Janzen, 2008). Consequently, whilst developmental thermal plasticity of offspring is mediated by thermal plasticity of mothers (e.g., behavioural plasticity) in all reptiles, the interaction between plasticity of a female and her offspring is important throughout embryonic development in viviparous species, particularly in those species that are placentotrophic (e.g., Wapstra, 2000). Viviparous species, therefore, offer an interesting alternative context within which to investigate thermal developmental

plasticity, especially the complex interactions between plastic traits of mothers and their developing offspring (Noble *et al.*, 2018).

In this thesis I integrated long-term data, manipulative laboratory experiments and simulation modelling to investigate intraspecific variation in patterns of thermal developmental plasticity, using the placentotrophic viviparous lizard *Niveoscincus ocellatus*. In this general discussion, I will outline how my research has advanced key research areas relating to thermal developmental plasticity, with a focus on phenology and sex determination, and will suggest areas where more research is needed. Specifically, I will focus on three main areas in which I believe my thesis has made a significant contribution: 1) patterns of thermal developmental plasticity, among and within populations, 2) the adaptive significance of thermal developmental plasticity, and 3) the ecological and evolutionary consequences of patterns of thermal developmental plasticity.

1) Patterns of thermal developmental plasticity among and within populations

Populations from more variable environments are predicted to have greater adaptive plasticity than those from more stable environments, providing that the environment provides reliable cues of optimal phenotypes (Ghalambor *et al.*, 2007; Uller *et al.*, 2013), and the costs of plasticity do not outweigh the benefits (Uller, 2008; Angilletta, 2009). Patterns of plasticity are likely to vary between the core of a species' range and its margins because populations have evolved in different selective environments and have different biogeographic and demographic histories (Valladares *et al.*, 2014). A growing body of empirical research has demonstrated that plastic responses can differ among climatically distinct populations and species (e.g., Pigliucci *et al.*, 1995; Balaguer *et al.*, 2001; Valladares *et al.*, 2007; Husby *et al.*, 2010; Uller *et al.*, 2011; Rutschmann *et al.*, 2015; Quéméré *et al.*, 2018). Despite this, for the majority of species, we know relatively little about how populations differ in their plastic responses across landscapes.

One of the reasons for this lack of understanding is the paucity of long-term studies that track more than a single population across years. The absence of multi-population long-term datasets makes it difficult to identify differences in

phenotypically plastic responses among populations, especially where these responses are subtle, or are not amenable to laboratory experiments. Long-term studies also enable the estimation of within-population variation in traits and, often, facilitate the construction of pedigrees (Pemberton, 2008). This can enable researchers to partition phenotypic variation and reaction norms into environmental and heritable components under an animal model framework (Henderson, 1950).

In my thesis I addressed these considerations using a long-term dataset, tracking two climatically distinct populations of the viviparous skink, *Niveoscincus ocellatus*, across 16 years. In Chapter 2, I used this long-term dataset to explore differences in the population-mean thermal reaction norms of two phenotypically plastic traits (phenology and offspring sex) between populations. I showed that these populations share a similar phenological reaction norm, but that the effect of temperature on offspring sex differed substantially between populations. In Chapter 5, I used an animal model framework to investigate the level of among-individual variation in thermal reaction norms of phenology within these populations. Few studies have investigated the degree to which phenotypic plasticity varies among individuals within populations (but see Ljungström *et al.*, 2015; McGaugh *et al.*, 2010) and fewer still have compared how populations differ in the magnitude of among-individual variation within them (but see Husby *et al*., 2010; Porlier, *et al.*, 2012; Westneat *et* al., 2014; Quéméré *et al.*, 2018). I showed that, even where populations share a common mean-level reaction norm, the degree of among-individual variation within them can differ substantially (Chapter 5) which is likely to have consequences for how they will respond to changed conditions. Further research, making use of a similar, animal model approach, would help to establish whether this pattern is common among species and to assess the degree to which plastic responses are heritable among populations. Bivariate animal models can also be used to investigate the covariance between traits (Kruuk, 2004), and future research on this, and other systems, should focus on the covariance among traits to investigate, for instance, whether species or populations are characterised by general patterns of phenotypic plasticity among traits (i.e., whether populations are characterised by being generally plastic for a range of traits). While these approaches are now not new, logistical constraints (for example the dearth of long-term studies) have prevented

ecologists from taking up this challenge in more than a few taxa (e.g., Husby *et al*., 2010).

One interesting avenue would be to investigate whether other components of the reproductive cycle (e.g., vitellogenesis or ovulation) are advancing at rates similar to those of parturition dates. Differences in the magnitude of the effect of warming climates on these phenological events would lead to a change in overall developmental rates, which may have consequences for offspring development, individual fitness and, therefore, on population viability (While *et al.*, 2015). By integrating long-term field studies with manipulative experiments we can also test hypotheses of the adaptive benefits of plasticity and can characterise reaction norms across, and beyond the range of temperatures experienced in the wild.

Long-term studies also enable researchers to identify subtle plastic responses, which might be missed in short-term studies or where manipulative experiments are difficult to perform. For instance, the comparatively subtle effect of temperature on offspring sex in a lowland population of *N. ocellatus* I show in this thesis (Chapters 2 & 3) would not have been identified in a short-term study. In the absence of long-term data, covering multiple populations, researchers must turn to theoretical approaches to identify likely patterns of intraspecific variation in patterns of plasticity. These approaches, however, require solid conceptual understanding of the factors that have favoured the evolution of plasticity among populations. For instance, Pen *et al.* (2010) demonstrated that intraspecific divergence in sex determining systems in *N. ocellatus* resulted from differences between populations in the sex–specific benefits of early birth arising from differences between sites in the long-term mean and interannual variation in environmental temperatures during the gestation period (see Chapter 6). Thus, this conceptual model links the two thermally plastic traits I focussed on in this thesis: parturition phenology and sex determination. In Chapter 6, I presented an individual-based evolutionary simulation model explaining the evolution of TSD and GSD across climatic contexts using Pen *et al.*'s (2010) conceptual framework. Using this method, I made a number of significant advances on the results found by Pen and colleagues. I used my results to predict the likely distribution of sex determination systems across the full range of *N. ocellatus*. I showed that: i) patterns of sex determination vary among locations, depending on local climatic variables; ii) that

selection for TSD peaks at intermediate temperatures, rather than rising with long-term mean temperatures, which is due to the sex-specific benefit of early birth peaking at these intermediate temperatures; and iii) that transitions between sex determination systems are possible in both directions – that is, both from GSD to TSD, and from TSD to GSD via the evolution of novel sex determining elements. Combined, these results demonstrate that, within populations, sex-determining systems can alternate along environmental gradients. Importantly, this is possible whether environmental conditions vary geographically (i.e., sex determination varies among populations; see below), or temporally (i.e., sex determination varies over time within locations as climatic conditions change). This alternating pattern along environmental gradients has consequences for our understanding of the evolution of sex determination systems and other phenotypically plastic traits.

The results I present in Chapter 6, coupled with those from Chapters 2 and 3 and with earlier work on this species (Pen *et al.,* 2010) confirm that sex determination systems may be labile (see Janzen & Paukstis, 1991a; Ezaz *et al.*, 2009a; 2009b). Indeed, TSD and GSD systems should, rightly, be considered as the extremes of a continuum (e.g., Sarre *et al.*, 2004; Radder *et al.*, 2008; Grossen *et al.*, 2011) rather than as fundamentally different modes (e.g., Valenzuela *et al.*, 2003). Thus, my results might help to explain divergence in sex determination systems among closely related taxa. Indeed, in taxa known to be labile for sex determination, sex determination modes (GSD, TSD and mixed systems) may be constantly in flux over evolutionary timescales as climatic conditions change, and transitions can occur in either direction. TSD may, therefore, be much more common than is generally realised (at least in those species in which temperature has sex-specific fitness effects). Further research using an individual-based evolutionary simulation approach may help to predict which other species, or populations within them, might have TSD and, therefore, should be investigated to identify whether TSD is present. Importantly, while my approach has identified the likely distribution of sex determination systems across the landscape in *N. ocellatus*, it needs to be validated with data from additional populations. Characterising the genetic mechanisms underlying sex determination systems in this species would be an extremely valuable next step (see Hill et al. 2018 for the first step towards this goal). Hill *et al.* (2018) found that the divergence in sex determination systems in *N. ocellatus* may have been facilitated by subtle genetic

differences. Increased understanding of how patterns of phenotypic plasticity should be expected to vary across the landscape will enhance our ability to predict how species will respond to altered climatic conditions. In Chapter 6, I have shown that researchers should be cautious of defining species as being homogeneous for sex determination system if results are based on single populations (Chapters 2 & 6), or from populations that experience dissimilar climates (Chapter 6).

What role do maternal effects play in mediating these between-population differences in sex determination? Early developmental responses will be affected by a complex range of parental (especially maternal) environmental effects, which can have substantial impact on offspring development. This is particularly true of viviparous species. Viviparous species differ from most oviparous species, in that females are able to exert some control over developing offspring throughout gestation. By altering basking behaviour, females may, therefore be able to buffer offspring against the effects of temperature if environmental conditions change during embryonic development (see, for example Cadby *et al.*, 2014 and Caldwell *et al.*, 2017). Consequently, thermoregulatory behaviour may be obscuring the extent to which temperature has an effect on offspring sex in *N. ocellatus*. To date, however, the estimates of the effects of environmental temperature on offspring sex in *N. ocellatus* have been based on long-term correlative field data and on experiments in the laboratory in which pregnant females have been provided with varying opportunities to bask. Further experiments, holding pregnant females at constant temperatures, would be helpful in characterising the strength of the effect of temperature on offspring sex in this species by disentangling the physiological responses of developing offspring from the behavioural decisions of their mothers. Further research using incubator experiments should focus on unrepresented reptile taxa. Few studies have used this approach to investigate the effect of constant developmental temperatures on viviparous squamates (see supplementary information from Noble *et al.*, 2018) but this technique may help in identifying whether other viviparous species have TSD.

2) The adaptive significance of thermal developmental plasticity

Phenotypic plasticity can be advantageous where it enables organisms to optimise phenotypes across a range of environmental conditions. Plasticity, therefore, is likely to be adaptive where environmental conditions are variable and no single phenotype is optimal under all conditions (Ghalambor *et al.*, 2007). For phenotypes that become fixed (e.g., sex in species with Temperature-sensitive sex determination (TSD)), environmental conditions at the time of fixation must also reliably predict the optimal phenotype later in life (van Kleunen & Fischer, 2005; Wong & Ackerly, 2005). Where conditions are variable, but optimal phenotypes are less predictable, however, adaptive 'coin-tossing' ('bet-hedging'), may be more advantageous (Kaplan & Cooper, 1984; Marshal & Uller, 2007; Uller, 2008; Uller *et al.*, 2013). Plastic responses cannot, however, be assumed to be adaptive because altered phenotypes may result from physical or physiological constraints imposed by the environment (transmissive effects). Furthermore, where multiple traits are affected by an environmental variable (e.g., temperature in ectotherms) adaptively plastic responses may be constrained by trade-offs where there are concomitant effects on non-adaptive plastic traits.

In Chapter 4, I used a combination of field data and a manipulative study to show that date of birth has important effects on fitness in *N. ocellatus*. Specifically, I demonstrated that offspring born earlier as a result of high temperatures during gestation had increased growth over the first winter of life, but there was no effect on offspring survival, nor was there an effect of developmental temperature on the incidence of developmental abnormalities. This finding was consistent with previous work in this species and suggests limited selection on offspring birth date (Uller et al., 2011). Importantly, the increase in growth I showed has implications for the adaptive significance of TSD in this species (see Chapter 6).

Plastic responses will not only be dependent on the nature of fitness trade-offs, but also by the fact that a given trait is likely to be influenced by multiple intrinsic or extrinsic variables. However, the possibility that the multi-factorial complexities of natural systems may obscure results if multiple factors interact is often overlooked. Manipulative experimental studies, especially when they are integrated with field data, are invaluable for disentangling the effects of multiple, possibly interacting

variables that might affect the expression of phenotypes. In Chapter 3, I investigated whether the resources available to females affected sex allocation, phenology and offspring characteristics independently of, in addition to, or in interaction with a temperature effect. Whilst resource availability is a likely candidate for a factor that might affect phenology (e.g., because females may alter basking behaviour) or sex (Charnov-Bull, 1977), I showed that it did not have an effect on either dates of birth or offspring sex in this species. However, resource availability is not the only environmental factor that might alter these traits, and further research would be useful in identifying other potential factors and testing whether they are important. In *N. ocellatus*, any environmental variable that alters female basking behaviour may have an effect on sex allocation. For instance, females may bask less when they perceive greater predation risks (Greene, 1988) and this may affect sex ratios in viviparous species with TSD (Robert & Thompson, 2007). Similarly, predation risk may affect nest site choice in oviparous species with TSD and have similar consequences (Spencer & Thompson, 2003). Alternatively, predation risk may not affect pregnant females' basking behaviour if they prioritise thermoregulation over risk aversion to maintain optimal developmental temperatures for their offspring (e.g., Lorioux *et al.*, 2013). Indeed, the operational sex ratio (OSR) itself may affect basking behaviour if, for example, males harass females (While & Wapstra, 2009).

3) The ecological and evolutionary consequences of thermal developmental plasticity in a changing climate

Phenotypic plasticity is an important mechanism by which populations and species may be able to respond to altered climatic conditions, either by enabling populations to persist in altered conditions or by facilitating their colonisation of new sites (Matesanz *et al.*, 2010; Nicotra *et al.*, 2010). In predicting how species will be affected by climate change we need to take account of whether patterns of plasticity vary both among and within populations, and how this may drive or constrain evolutionary responses. In Chapter 5, I demonstrated that birth dates in *N. ocellatus* are similarly affected by environmental temperature in climatically distinct populations, and will advance to the same extent as climates warm.

Altered phenologies are one of the most often predicted and observed consequences of warming climates (e.g., Parmesan & Yohe, 2003; Root *et al.*, 2003; Parmesan, 2007; Charmantier *et al.*, 2008; Lane *et al.*, 2012; While & Uller, 2014; Ge *et al.*, 2016; Cohen *et al.*, 2018) and can have significant positive or negative consequences for population persistence (Charmantier *et al.*, 2008; Lane *et al.*, 2012). Predicting whether populations are likely to benefit or suffer from altered phenologies, however, requires an understanding of their ecological consequences. In Chapter 4, I showed that advancing birth dates are more likely to have positive than negative effects on population persistence because offspring growth is enhanced by early birth. This positive effect is in contrast to some predictions that have suggested that rising temperatures will have negative effects on population persistence in reptiles (e.g., Sinervo *et al.*, 2010). Importantly, however, these positive effects are only likely if temperatures do not warm to the extent that normal development is compromised. Indeed, as climate variability increases, extreme events are likely to increase in both severity and frequency (Collins *et al.*, 2013) and mean temperatures are likely to rise beyond those I have investigated. Further research, investigating the effects of very high (and variable) temperatures is needed to assess the effects of these extremes. Furthermore, understanding how temperature affects populations, especially those at the margins of a species' distribution, will facilitate or constrain the capacity of species to colonise new locations (Valladares *et al*., 2014). For instance, whether populations of *N. ocellatus* will colonise higher elevations as climates warm.

Even where populations share similar mean-level reaction norms, however, they may differ in their degree of among-individual variation. In Chapter 5, I showed that, despite having similar mean-level reaction norms, populations of *N. ocellatus* differ substantially in the their degree of among-individual variation. Variation among individuals in the effect of environmental conditions on phenotypically plastic traits is an important consideration and can have substantial ecological consequences (Bolnick, *et al.*, 2011), especially in extreme conditions. Where variation among individuals is low, adverse conditions are likely to affect all individuals within a population similarly. High among-individual variation may therefore buffer populations against the effects of extreme events. Variation among individuals within populations, and the degree to which this variation is heritable, is also important in determining the capacity of populations to evolve in response to changed selective

pressures. Therefore, further efforts should be made to integrate both among and within population variation in patterns of plasticity into species distribution models in the future. Furthermore, to gain a better understanding of patterns of plasticity among and within populations it is also important that we understand the mechanisms by which plastic traits are expressed, and their genetic underpinnings. Further research should, therefore, focus on gaining a greater understanding of the mechanistic underpinnings of phenotypically plastic traits.

In this thesis I have shown that phenotypic responses may vary among populations even in fundamental characteristics, such as modes of sex determination (Chapter 2). The adaptive significance of sex determination systems in reptiles is a topic that has attracted significant research interest (e.g., Charnov & Bull, 1977; Janzen & Paukstis, 1991a; Burke, 1993; Shine, 1999; Janzen & Phillips, 2006; Pen *et al*., 2010). Species with TSD are generally considered to be at high risk from warming climates, because of the potential for sex ratios to become skewed as climates increasingly diverge from historical patterns (Janzen, 1994; Walther *et al.*, 2002; Mitchel & Janzen, 2010). I suggested, however, that populations of *N. ocellatus* with TSD are not at high risk because of both the direction of the response (female skews, relative to the long-term sex ratio, at high temperatures) and because the effect is comparatively weak, in contrast to many other species with TSD (i.e., sex ratios are not skewed to the extent that either 100% males or females are produced). Sex-determining mechanisms have been identified in less than 5% of known taxa, and taxonomic coverage is strongly biased towards oviparous species, particularly turtles (While *et al*., 2018). This is because thermal effects on offspring sex are comparatively easy to manipulate in oviparous species when compared to viviparous species, and it is from these types of experiments that strong (i.e., 100% male or female litters) effects are most often reported (Janzen and Paukstis, 1991b; Congdon *et al.*, 1995; Inamdar *et al.*, 2012; Gomez-Saldarriaga *et al.*, 2016; Marco *et al.*, 2017). Even in species with comparatively steep reaction norms (i.e., 100% males or females on either side of a well-defined pivotal temperature), however, there is increasing evidence that when incubation temperatures fluctuate, sex ratios are rarely skewed to the extent we may predict from the artificially stable environment of laboratory incubators (e.g., Bull, 1985; Georges *et al.*, 1994; Les *et al.*, 2007; Paitz *et al.*, 2010; Neuwald & Valenzuela, 2011; Bowden *et al.*, 2014; Carter *et al.*, 2017).

Thus, thermal effects on sex determination are difficult to detect in wild populations, especially in the 20-30% that are viviparous.

Some types of species distribution models have the capacity to incorporate patterns of among-population variability and adaptive evolution into predictions of the effect of altered climatic conditions on species (e.g., mechanistic species distribution models; Kearney & Porter, 2009). In practice, however, modellers often fail to account for phenotypically plastic responses. Even where they do, it is rare for them to incorporate inter-population variation in patterns of plasticity (but see Valladares *et al.*, 2014). These models, however, need to be parameterised with data from natural systems, or base estimates of patterns on a firm theoretical base (Lawson *et al.*, 2015; McLean *et al.*, 2016), as I have provided for *N. ocellatus*, here. Understanding the consequences of population-specific patterns of phenotypic plasticity for species requires that we have some idea of how patterns of plasticity and the strength of plastic responses vary among populations in the present, and how selective pressures will be changed under altered climatic conditions (see above). In Chapter 6, I addressed these challenges using a simulation modelling approach to investigate how selection for TSD will be affected by warming climates across the range of *N. ocellatus*. Understanding patterns of intraspecific variation in phenotypic plasticity, both within and among populations is important for understanding ecological change in response to climate change both on short-term ecological and on long-term evolutionary time-scales. Indeed, altered climatic conditions as a result of ongoing environmental change will have fundamental implications for selection regimes and contribute significantly to evolutionary change. Ecological and evolutionary processes are ultimately related, because ecological processes feedback to influence evolutionary change and these evolutionary changes (both plasticity and genetic) are fundamental in how species will respond to global challenges.

Concluding remarks

Fifteen years ago, Lesley Hughes published a seminal paper in which she pointed out that Australia lacks long-term datasets, specifically studies that monitor phenologies, such as those which have permitted Northern Hemisphere biologists to detect effects of warming climates on species (Hughes, 2003). In this paper, Hughes challenged

Australian researchers to place more focus on long-term studies, especially for taxa likely to be impacted by global climate change. My thesis emphasises the value of long-term studies in characterising how populations will respond to changed environmental conditions. In this thesis I used a long-term dataset, tracking two climatically-distinct populations of *N. ocellatus* over 16 years, to investigate the climatic determinants of patterns of phenotypic plasticity across the landscape of Tasmania, and to predict how species will be affected by altered climatic conditions. As climates continue to warm and become more variable globally, it is more important than ever that we understand the ecology and evolution of species and how they respond to change.

References

Andrews, R.M., Mathies, T., Warner, D.A. & Mathies, T.O.M. (2009). Effect of incubation temperature on morphology, growth, and survival of juvenile Sceloporus undulatus. *Herpetological Monographs*, **14**: 420–431.

Angilletta, M. (2009). *Thermal adaptation: a theoretical and empirical analysis*. Oxford: Oxford University Press.

Balaguer, L., Martínez-Ferri, E., Valladares, F., Pérez-Corona, M.E., Baquedano, F.J., Castillo, F.J. & Manrique, E. (2001). Population divergence in the plasticity of the response of *Quercus coccifera* to the light environment. *Functional Ecology*, **15**: 124-135.

Beldade, P., Mateus, A.R.A. & Keller, R.A. (2011) Evolution and molecular mechanisms of adaptive developmental plasticity. *Molecular Ecology*, **20**: 1347-1363.

Bolnick, D.I., Amarasekare, P., Araújo, M.S., Bürger, R, Levine, J.M., Novak, M, Rudolf, V.H.W., Schreiber, S.J., Urban, M.C. & Vasseur, D. (2011) Why intraspecific variation matters in community ecology. *Trends in Ecology and Evolution*, 26: 183-192.

Bowden, R.M., Carter, A.W. & Paitz, R.T. (2014). Constancy in an inconstant world: moving beyond constant temperatures in the study of reptilian incubation. *Integrative and Comparative Biology*, **54**: 830-840.

Boyle, M., Schwanz, L., Hone, J. & Georges, A. (2016). Dispersal and climate warming determine range shift in model reptile populations. *Ecological Modelling*, **328**: 34–43.

Brommer, J.E. (2013) Phenotypic plasticity of labile traits in the wild. *Current Zoology*, **59**: 485-505

Bull, J.J. (1985). Sex-ratio and nest temperature in turtles – comparing field and laboratory data. *Ecology*, **66**: 1115-1122.

Burke, R.L. (1993). Adaptive value of sex determination mode and hatchling sex-ratio bias in reptiles. *Copeia*, **3**: 854-859.

Cadby, C.D., Jones, S.M. & Wapstra, E. (2014). Geographical differences in maternal basking behaviour and offspring growth rate in a climatically widespread viviparous reptile. *Journal of Experimental Biology*, **217**: 1175-1179

Caldwell, A.J., While, G.M. & Wapstra, E. (2017). Plasticity of thermoregulatory behaviour in response to the thermal environment by widespread and alpine reptile species. *Animal Behaviour*, **132**, 217-227.

Carter, A.W., Bowden, R.M. & Paitz, R.T. (2017). Seasonal shifts in sex ratios are mediated by maternal effects and fluctuating incubation temperatures. *Functinoal Ecology*, **31**: 876-884.

Chapman, D.S., Scalone, R., Stefanic, E. & Bullock, J.M. (2017). Mechanistic pecies distribution modelling reveals a niche shift during invasion. *Ecology*, **98**, 1671-1680.

Charmantier, A., McCleery, R.H., Cole, L.R., Perrins, C., Kruuk, L.E.B. & Sheldon, B.C. (2008). Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science*, **320**: 800-803.

Charnov, E.L. & Bull, J. (1977). When is sex environmentally determined? *Nature*, **266**: 828–830.

Chevin, L.M. & Lande, R. (2010). Adaptation, plasticity and extinction in a changing environment: towards a predictive theory. *PLOS Biology*, **8**, e1000357.

Chevin, L.M., Collins, S. & Lefevre, F. (2013). Phenotypic plasticity and evolutionary demographic responses to climate change: taking theory out to the field. *Functional Ecology*, **27**, 966-979.

Cohen, J.M., Lajeunesse, M.J. & Rohr, J.R. (2018). A global synthesis of animal phenological responses to climate change. *Nature Climate Change*, **8**, 224-228.

Collins, M., Knutti, R., Arblaster, J., Dufresne, J.-L., Fichefet, T., Friedlingstein, P., Gao, X., Gutowski, W.J., Johns, T., Krinner, G. *et al.* (2013). Long-term Climate Change: Projections, Commitments and Irreversibility. In: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V. & Midgley, P.M. (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Congdon, J.D., Fischer, R.U. & Gatten, R.E. (1995). Effects of incubation temperatures on characteristics of American alligators. *Herpetologica*, **51**: 497-504.

Deeming, D.C. (2004). Post-hatching phenotypic effects of incubation in reptiles. In: *In Reptilian Incubation: Environment, Evolution and Behaviour* (ed. Deeming, D.C.). Nottingham University Press., pp. 229–252.

Dillon, M.E., Wang, G. & Huey, R.B. (2010). Global metabolic impacts of recent climate warming. *Nature*, 467, 704–706.

Doody, J., Guarino, E., Georges, A., Corey, B., Murray, G. & Ewert, M. (2006). Nest site choice compensates for climate effects on sex ratios in a lizard with environmental sex determination. *Evolutionary* Ecology, **20:** 307-330.

Duputie, A., Rutschmann, A., Ronce, O., Chuine, I. (2015). Phenological plasticity will not help all species adapt to climate change. *Global Change Biology*, **21**, 3062-3073.

Ezaz, T., Quinn, A.E., Sarre, S.D., O'Meally, D., Georges, A. & Graves, J.A.M. (2009a). Molecular marker suggests rapid changes of sex-determining mechanisms in Australian dragon lizards. *Chromosome Research*, **17**: 91-98.

Ezaz, T, Sarre, S.D., O'Meally, D., Graves, J.A.M, Georges, A. (2009b). Sex chromosome evolution in lizards: independent origins and rapid transitions. *Cytogenetic and Genome Research*, **127**: 249-260.

Fusco, G. & Minelli, A. (2010). Phenotypic plasticity in development and evolution: facts and concepts. *Philosophical Transactions of the Royal Society B- Biological Sciences*, **365**: 547-556.

Ge, Q.S., Wang, H.J., Rutishauser, T. & Dai, J.H. (2016). Phenological responses to climate change in China: a meta-analysis. *Global Change Biology*, **21:** 265-274.

Georges, A., Limpus, C. & Stoutjesdijk, R. (1994). Hatchling sex in the marine turtle *Caretta caretta* is determined by proportion of development at a temperature, not daily duration of exposure. *Journal of Experimental Zoology*, **270**: 432-444.

Ghalambor, C.K., McKay, J.K., Carroll, S.P. & Reznick, D.N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, **21**: 394-407.

Gomez-Saldarriaga, C., Valenzuela, N. & Ceballos, C.P. (2016). Effects of incubation temperature on sex determination in the endangered Magdalena River turtle, *Podocnemis lewyana*, **15**: 43-53.

Greene, H. W. 1988. Anti-predator mechanisms in reptiles. In: Grans, C. & Huey, R.B. (eds), *Biology of Reptilia 16, Ecology B: Defense and Life History*. Alan R. Liss, New York, pp. 1–152.

Grossen, C., Neuenschwander, S. & Perrin, N. (2011) Temperature-dependent turnovers in sex-determination mechanisms: a quantitative model. *Evolution*, **65**: 64-78.

Hawkes, L.A., Broderick, A.C., Godfrey, M.H. & Godley, B.J. (2007). Investigating the potential impacts of climate change on a marine turtle population. *Global Change Biology*, **13**: 923-932.

Henderson, C.R. (1950). Estimation of genetic parameters. *Annals of Mathematical Statistics*, **21**: 309-310.

Hill, P.L., Burridge, C.P., Ezaz, T., Wapstra, E. (2018). Conservation of sex-linked markers among conspecific populations of a viviparous skink, *Niveoscincus ocellatus*, exhibiting genetic and temperature-dependent sex determination. *Genome Biology and Evolution*, **10**: 1079-1087.

Hoffmann, A.A. & Sgro, C.M. (2011) Climate change and evolutionary adaptation. *Nature*, **470**: 479-845

Huey, R.B., Kearney, M.R., Krockenberger, A., Holtum, J.A.M., Jess, M. & Williams, S.E. (2012). Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Philosophical Transactions of the Royal Society- B Biological Sciences*, **367**: 1665–1679.

Hughes, L. (2003) Climate change and Australia: trends and impacts. *Austral Ecology*, **28**: 423-443.

Husby, A., Nussey, D.H., Visser, M.E., Wilson, A.J., Sheldon, B.C., Kruuk, L.E.B. (2010). Contrasting patterns of phenotypic plasticity in reproductive traits in two great tit (*Parus major*) populations. *Evolution*, **64**, 2221-2237.

Inamdar, L.S., Vani, V. & Seshagiri, P.B. (2012). A tropical oviparous lizard, *Calotes veriscolor*, exhibiting a potentially novel FMFM pattern of temperature-

dependent sex determination. *Journal of Experimental Zoology Part A-Ecological Genetics and Physiology*, **317A**: 32-46

Janzen, F.J. (1994) Climate change and temperature-dependent sex determination in reptiles. *Proceedings of the National Academy of Science*s, **91**: 7487-7490.

Janzen, F.J. & Paukstis, G.L. (1991a) A preliminary test of the adaptive significance of environmental sex determination in reptiles. *Evolution*, **45**: 435-440.

Janzen, F.J. & Paukstis, G.L. (1991b) Environmental sex determination in reptiles – ecology, evolution, and experimental design. *Quarterly Review of Biology*, **66**: 149-179.

Janzen, F.J. & Phillips, P.C. (2006). Exploring the evolution of environmental sex determination, especially in reptiles. *Journal of Evolutionary Biology*, **19**: 1775-1784.

Kaplan, R.H. & Cooper, W.S. (1984). The evolution of developmental plasticity in reproductive characteristics: an application of the "adaptive coin-flipping" principle. *American Naturalist*, **123**: 393-410.

Kearney, M. & Porter, W. (2009) Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. *Ecology Letters*, **12**: 334-350.

Kovach-Orr, C. & Fussmann, G.F. (2013). Evolutionary and plastic rescue in multitrophic model communities. *Philosophical Transactions of the Royal Society B – Biological Sciences*, **368**, 20120084.

Kruuk, L. (2004) Estimating genetic parameters in natural populations using the 'animal model'. *Philosophical Transactions of the Royal Society of London B – Biological Sciences*, **359**: 873-890.

Lane, J.E., Kruuk, L.E.B., Charmantier, A., Murie, J.O. & Dobson, F.S. (2012). Delayed phenology and reduced fitness associated with climate change in a wild hibernator. *Nature*, **489**: 554-+.

Lawson, C.R., Vindenes, Y., Bailey, L. & van der Pol, M. (2015) Environmental variation and population responses to global change. *Ecology Letters*, **18**, 724-736.

Les, H.L., Paitz, R.T. & Bowden, R.M. (2007). Experimental test of the effects of fluctuating incubation temperatures on hatchling phenotype. *Journal of Experimental Zoology Part A – Ecological Genetics and Physiology*, **370A**: 274-280.

Ljungström, G., Wapstra, E. & Olsson, M. (2015) Sand lizard (*Lacerta agilis*) phenology in a warming world. *BMC Evolutionary Biology* **15**, 206.

Lorioux , S., Lisse, H., & Lourdais, O. (2013). Dedicated mothers: predation risk and physical burden do not alter the thermoregulatory behaviour of pregnant vipers. *Animal Behaviour*, **86**: 401-408.

Matesanz, S., Gianoli, E & Valladares, F. (2010). Global climate change and the evolution of phenotypic plasticity in plants. *Annals of the New York Academy of Sciences*, **1206**: 35-55.

Marco, M.V.P, Leiva, P., Iungman, J.L., Simoncini, M.S. & Pina, C.I. (2017). New evidence characterising temperature-dependent sex determination in broad-snouted caiman, *Caiman latirostris*, **12**: 78-84.

Marshall, D.J. & Uller, T. (2007) When is a maternal effect adaptive? *Oikos*, **116**: 1957-1963.

McGaugh, S.E., Schwanz, L.E., Bowden, R.M., Gonzalez, J.E. & Janzen, F.J. (2010). Inheritance of nesting behaviour across natural environmental variation in a turtle with temperature-dependent sex determination. *Proceedings of the Royal Society B – Biological Sciences*, **277**, 1219-1226.

McLean, N., Lawson, C.R., Leech, D.I. & van der Pol, M. (2016). Predicting when climate-driven phenotypic change affects population dynamics. *Ecology Letters*, **19**: 595-608.

Mitchell, N.J., Kearney, M.R., Nelson, N.J. & Porter, W.P. (2008). Predicting the fate of a living fossil: how will global warming affect sex determination and hatchling phenology in tuatara? *Proceedings of the Royal Society of London B: Biological Sciences* **275**: 2185–2193.

Mitchell, N.J. & Janzen, F.J. (2010) Temperature-dependent sex determination and contemporary climate change. *Sexual Development*, **4**: 129-140.

Morjan, C. L. (2003). Variation in nesting patterns affecting nest temperatures in two populations of painted turtles (*Chrysemys picta*) with temperature-dependent sex determination. *Behavioral Ecology and Sociobiology*, **53:** 254-261.

Neuwald, J.L. & Valenzuela, N. (2011). The lesser known challenge of climate change: thermal variance and sex-reversal in vertebrates with temperature-dependent sex determination. *PLOS ONE*, **6**: e18117.

Nicotra, A.B., Atkin, O.K., Bonser, S.P., Davidson, A.M., Finnegan, E.J., Mathesius, U., Poot, P., Purugganan, M.D., Richards, C.L., Valladares, F. *et al.* (2010) Plant phenotypic plasticity in a changing climate. *Trends in Plant Science*, **15**: 684-692.

Noble, D.W.A., Stenhouse, V. & Schwanz, L.E. (2018) Developmental temperatures and phenotypic plasticity in reptiles: a systematic review and meta-analysis. *Biological Reviews*, **93**: 72-97.

Paitz, R.T., Gould, A.C., Holgersson, M.C.N. & Bowden, R.M. (2010). Temperature, phenotype, and the evolution of temperature-dependent sex determination: how do natural incubations compare to laboratory incubations? *Journal of Experimental Zoology Part B – Molecular and Developmental Evolution*, **314B**: 86-93.

Parmesan, C. (2007). Influences of species, latitudes and methodologies on estimates of phenological response to global warming. *Global Change Biology*, **13**, 1860–1872.

Parmesan, C. & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, **421**, 37–42.

Pemberton, J. (2008). Wild pedigrees: the way forward. *Proceedings of the Royal Society B – Biological Sciences*, **275**: 613-621.

Pen, I., Uller, T., Feldmeyer, B., Harts, A., While, G.M. & Wapstra, E. (2010). Climate-driven population divergence in sex-determining systems. *Nature*, **468**, 436.

Pigliucci, M., Whitton, J. & Schlichting, C.D. (1995). Reaction norms of Arabdopsis. 1. Plasticity of characters and correlations across water, nutrient and light gradients. *Journal of Evolutionary Biology*, **8**: 421-438.

Porlier, M., Charmantier, A., Bourgault, P, Perret, P., Blondel, J. & Garant, D. (2012) Variation in phenotypic plasticity and selection patterns in blue tit breeding time: between- and within- population comparisons. *Journal of Animal Ecology*. **81**: 1041-1051.

Quéméré, E., Gaillard, J.M., Galan, M., Vanpé, C., David, I., Pellerin, M., Kjellander, P., Hewison, A.J.M. & Pemberton, J.M. (2018) Between-population differences in the genetic and maternal components of body mass in roe deer. *BMC Evolutionary Biology*, **18**: 39.

Radder, R.S., Quinn, A.E., Georges, A, Sarre, S.D. & Shine, R. (2008) Genetic evidence for co-occurance of chromosomal and thermal sex-determining systems in a lizard.

Reed, T.E., Schindler, D.E. & Waples, R.S. (2011). Interacting effects of phenotypic plasticity and evolution on population persistence in a changing climate. *Conservation Biology*, 25, 56–63.

Robert, K.A. & Thompson, M.B. (2007). Is basking opportunity in the viviparous lizard, *Eulamprus tympanum*, compromised by the presence of a predator scent? *Journal of Herpetology*, **41**: 287-293.

Root. T.L., Price, J.T., Hall, K.R., Schneider, S.H., Rosenzweig, C. & Pounds, J.A. (2003). Fingerprints of global warming on wild animals and plants. *Nature*, **421**, 57–60.

Rutschmann, A., Miles, D.B., Le Galliard, J.-F., Richard, M., Moulherat, S., Sinervo, B. & Clobert, J. (2015) Climate and habitat interact to shape the thermal reaction norms of breeding and phenology across lizard populations. *Journal of Animal Ecology*, **85**: 457-466.

Sarre, S.D., Georges, A. & Quinn, A. (2004) The ends of a continuum: genetic and temperature-dependent sex determination in reptiles. *Bioessays*, **26**: 639-645.

Schwanz, L.E. & Janzen, F.J. (2008). Climate change and temperature‐dependent sex determination: can individual plasticity in nesting phenology prevent extreme sex ratios? *Physiological and Biochemical Zoology* **81,** 826-834.

Schwanz, L.E., Cordero, G.A., Charnov, E.I. & Janzen, F.J. (2016). Sex-specific survival to maturity and the evolution of environmental sex determination. *Evolution (N. Y).*, **70**: 329–341.

Seebacher, F., White, C.R. & Franklin, C.E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change*, **5**: 61–66.

Sinervo, B., Mendez-de-la-Cruz, F., Miles, D.B., Heulin, B., Bastiaans, E., Cruz, M.V. S., Lara-Resendiz, R., Martinez-Mendez, N., Calderon-Espinosa, M.L., Meza- Lazaro, R.N. *et al.* (2010) Erosion of lizard diversity by climate change and altered thermal niches. *Science*, **328**: 894-899.

Shine, R. (1999). Why is sex determined by nest temperature in many reptiles? *Trends in Ecology and Evolution*, **14**: 186–189.

Spencer, R.J. & Thompson, M.B. (2003) The significance of predation in nest site selection of turtles: an experimental consideration of macro- and microhabitat preferences. *Oikos*, **102**: 592-600.

Uller, T. (2008). Developmental plasticity and the evolution of parental effects. *Trends in Ecology and Evolution*, **23**: 432-438.Uller, T., While, G.M., Cadby, C.D., Harts, A., O'Connor, K., Pen, I. & Wapstra, E. (2011). Altitudinal divergence in maternal thermoregulatory behaviour may be driven by differences in selection on offspring survival in a viviparous lizard. *Evolution*, **65**, 2313-2324

Uller, T., Nakagawa, S. & English, S. (2013). Weak evidence for anticipatory parental effects in plants and animals. *Journal of Evolutionary Biology*, **26**: 2161-2170.

Valenzuela, N., Adams, D.C. & Janzen, F.J. (2003) Pattern does not equal process: Exactly when is sex environmentally determined? *American Naturalist*, **161**: 676-683.

Valladares, F., Gianoli, E. & Gómez, J.M. (2007). Ecological limits to plant phenotypic plasticity. *New Phytologist*, **176**: 749–763. Valladares, F., Matesanz, S., Guilhaumon, F., Araujo, M.B., Balaguer, L., Benito-Garzon, M., Cornwell, W., Gianoli, E., van Kleunen, M., Naya, D.E. *et al.* (2014). The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecology Letters*, **17**: 1351-1364.

van Kleunen, M. & Fischer, M. (2005) Constraints on the evolution of phenotypic plasticity in plants. *New Phytologist*, **166**: 49-60.

Vicenzi, N., Corbalan, V., Miles, D., Sinervo, B. & Ibarguengoytia, N. (2017). Range increment or range detriment? Predicting potential changes in distribution caused by climate change for the endemic high-Andean lizard *Phymaturus palluma*. *Biological Conservation*, **206**, 151-160.

Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin J.M., Hoegh-Guldberg, O. & Bairlein, F. (2002). Ecological responses to climate change. *Nature*, **416**: 389-395.

Warner, D.A. & Shine, R. (2008). The adaptive significance of temperature-dependent sex determination in a reptile. *Nature*, **451**: 566–568.

Wapstra, E. 2000. Maternal basking opportunity affects juvenile phenotype in a viviparous lizard. *Funct. Ecol.* **14**: 345-352.

West-Eberhard, M.J. (2003). *Developmental Plasticty and Evolution*. Oxford University Press, USA.

Westneat, D.F., Bokony, V., Burke, T., Chastel, O., Jensen, H., Kvalnes, T., Lendavi, A.Z., Liker, A., Mock, D., Schroeder, J. *et al*. (2014) Multiple aspects of plasticity in clutch size vary among populations of a globally distributed songbird. *Journal of Animal Ecology*, **83**: 876-887.

Wong, T.G. & Ackerly, D.D. (2005). Optimal reproductive allocation in annuals and an information constraint on plasticity. *New Phytologist*, **166**: 159-172.

While, G.M. & Wapstra, E. (2009) Snow skinks (*Niveoscincus ocellatus*) do not shift their sex allocation patterns in response to mating history. *Behaviour*, **146**: 1405-1422.

While, G.M. & Uller, T. (2014). Quo vadis amphibia? Global warming and breeding phenology in frogs, toads and salamanders. *Ecography*, **37**, 921-929.

While, G.M., Williamson, J., Prescott, G., Horvathova, T., Fresnillo, B., Beeton, N.J., Halliwell, B., Michaelides, S. & Uller, T. (2015). Adaptive responses to cool climate promotes persistence of a non-native lizard. *Proceedings of the Royal Society of London, B- Biological Sciences*. **282**: 20142638.

While, G.M., Noble, D.W.A., Uller, T., Warner, D.A., Du, W.-G., Riley, J.L. & Schwanz, L.E. (2018). Patterns of developmental plasticity in response to incubation temperature in reptiles. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, ***in press***.

Appendix:

An Experimental Test of Relatedness-Based Mate Discrimination in a Social Lizard

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Abstract

One of the major decisions individuals of many species make when deciding who to mate with is whether or not to inbreed. The prevailing theory is that individuals should avoid mating with closely related individuals because of the fitness costs associated with decreased offspring quality. However, theoretical work has suggested that levels of inbreeding depression need to be considerable for these costs to outweigh the possible, kin selected, benefits of inbreeding. While evidence for a preference for inbreeding exists in the literature, empirical results currently lag well behind theory. Here we conducted an experiment to examine mate discrimination and preference with respect to relatedness in a natural population of the family living lizard, *Liopholis whitii*. We show that both male and female *L. whitii* distinguish between related and unrelated individuals and, furthermore, that both sexes preferentially associate with more closely related members of the opposite sex. These results correspond closely with patterns of pairing observed in the wild where both males and females have been shown to choose mating pairs that are more closely related to them than expected by chance. Combined, our findings suggest that mate choice in this system may represent an active preference for mating with related individuals rather than a result of passive constraints associated with population viscosity.

Significance Statement

In species with long-term pair bonds, who to pair with is one of the most important decisions an organism will make. Much of the research on this topic focusses on avoiding individuals who are closely related, because mating with closely related individuals (e.g. inbreeding) decreases offspring quality. However, avoiding inbreeding may not always be the optimal strategy; it may actually pay to inbreed under certain conditions. Using a family living lizard that forms long-term pair bonds we show that males and females preferentially associate with more related potential mates when given a choice. These results add to a growing body of literature which suggests that partner choice with respect to relatedness may be the result of an active preference for more related individuals rather than a result of constraints on optimal partner choice.

Introduction

Mate choice is a fundamental process, which has the potential to drive the evolutionary and ecological trajectory of populations (Keller and Waller 2002; Charlesworth 2006). One of the major decisions individuals of many species face when deciding who to mate with is whether or not to inbreed. Because inbreeding has the potential to have a number of negative consequences for offspring fitness (e.g. through the presence of deleterious homozygous alleles or the loss of heterosis; Keller and Waller 2002; Charlesworth and Willis 2009) the prevailing theory has been that individuals should avoid mating with closely related individuals. In line with this, substantial empirical work across a range of taxa has shown that individuals often have a preference for unrelated partners (e.g. Bretman et al. 2009; Fitzpatrick and Evans 2014; Fitzpatrick et al. 2014; Liu et al. 2014). Where preferences for inbreeding have been observed, they tend to be attributed to constraints on mating opportunities or an inability to recognize kin rather than an active preference for related partners (Pusey and Wolf 1996; Keller and Arcese 1998; Oh 2011).

This focus on inbreeding avoidance ignores a number of key points. First, there are costs associated with avoidance of inbreeding such as those related to dispersal or missed opportunities for mating (Keller and Arcese 1998; Kokko and Ots 2006; Olson et al. 2012). Second, inbreeding and inbreeding depression are expressed in different generations. Therefore, selection against being inbred (as affected by inbreeding depression) does not necessarily correspond with selection for inbreeding avoidance (which also depends on the lifetime numbers of inbred and outbred offspring produced) (Reid et al. 2015). Finally, inbreeding may actually be beneficial, as it provides closely related individuals with additional breeding opportunities and because parents are more closely related to inbred offspring than they are to outbred offspring. Thus, in many cases individuals stand to gain substantial inclusive fitness benefits by choosing to mate with more closely related partners (Parker 1979; Smith 1979; Bateson 1983; Waser et al. 1986; Puurtinen 2011; Duthie and Reid 2015; Reid et al. 2015). Indeed, theoretical work suggests that levels of inbreeding depression need to be considerable for its costs to outweigh its possible, kin selected, benefits (Kokko and Ots 2006), thus inbreeding preference should be the rule rather than the exception. However, while evidence for a preference for inbreeding exists in the

literature (e.g. Kleven et al. 2005; Thünken et al. 2007; Langen et al. 2011; Wang and Lu 2011; Robinson et al. 2012), empirical results currently lag well behind the theory.

Here we examined the extent to which mate discrimination is influenced by genetic relatedness in a family living lizard, *Liopholis whitii*. *L. whitii* live in stable family groups consisting of a long-term socially monogamous male-female pair and their offspring, who delay dispersal and remain within their parents burrow system for a year sometimes two (Chapple and Keogh 2005; While et al. 2009). Previous research has shown that that social pairs are significantly more related to one another compared to a random pairing of individuals in the population (While et al. 2014). Furthermore, when undertaking extra-pair copulations (which account for 30% of offspring; While et al. 2009a, b, 2011), males and females breed with individuals that are significantly less related to themselves than their social partners (While et al. 2014). These results suggest that social partner choice in this system may be constrained by strong genetic structure at a local scale and that extra-pair mate choice acts as a mechanism to alleviate this constraint (While et al. 2014). However, there are several factors that suggest caution with this interpretation. First, levels of inbreeding depression at the population level are extremely low, and are not significantly different from zero, suggesting limited costs associated with inbreeding (While et al. 2014). Second, pair relatedness does not predict whether individuals undertake extra-pair copulations, suggesting that extra-pair paternity might not be a mechanism to compensate for pairing with a closely related individual (see also Wang and Lu 2011). Finally, although extra-pair offspring are significantly more heterozygous than within-pair offspring, there is no evidence that this heterozygosity is correlated with estimates of fitness (While et al. 2014). Additional work is therefore required to distinguish the extent to which pairing with closely related individuals is a result of passive constraints associated with population viscosity or is an active preference for mating with related individuals.

To address this we conducted an experiment during the mating season that allowed us to examine mate discrimination and preference with respect to relatedness for both males and females and to compare this to the patterns of pairing observed from our natural population of *L. whitii* (see While et al. 2014). We used a Y-maze choice design to determine a) if individuals discriminate between related and

unrelated individuals (measured via rate of tongue flicks) and b) if individuals exhibit preference for related, compared to unrelated, individuals (measured via time spent in association). Several predictions are possible, based on this design. First, we predict that there will be differences in tongue flick rates directed towards related and unrelated individuals (measured in each arm of the maze). Second, if the pairing with related individuals observed in the wild is a result of active choice for more closely related individuals, we predict that individuals will spend more time associated with more related individuals of the opposite sex compared to less related individuals. Alternatively, if pairing with a related individual is the result of constraints, then we predict that individuals will spend less time associated with more related individuals of the opposite sex or will spend equal amounts of time with both potential mates. Finally, as trade-offs between the benefits and costs of inbreeding are suggested to differ between males and females due to unequal patterns of reproductive investment (Pizzari et al. 2004; Kokko and Ots 2006; Reid et al. 2015), we predict that the responses will be stronger in females compared to males.

Methods

Study Species

Liopholis whitii is a medium-sized (up to 100 mm snout-vent length (SVL)) viviparous lizard found throughout a broad altitudinal (0–1600 m) and ecological (coastal heaths, grasslands, and forests) range in south-eastern Australia. We used *L. whitii* from the east coast of Tasmania, Australia (42°57′S, 147°88′E). In this population, males and females are sexually monomorphic, become reproductively mature at approximately 3 years and display an overall lifespan of 9–10 years (GMW unpublished data). Reproduction occurs annually, with mating during the austral spring (September–October) and gestation spanning 3–4 months (While et al. 2009a). Recent work from natural populations of *L. whitii* showed that there is substantial variation in relatedness within populations, creating opportunities for both inbreeding preference and avoidance (Chapple and Keogh 2005; While et al. 2014).

Field methods

We captured 71 adult lizards (SVL ≥ 75 mm) at the beginning of the Austral autumn (March) in 2008. All individuals were captured from an area of approximately 500m x 500m, using 'mealworming' and noosing techniques. Once captured, individuals were measured for weight (± 0.1 mg) and length (SVL and total length; ± 0.5 mm), and sex was determined via eversion of the hemipenes. Each individual had a portion of its tail clipped and stored in 70% ethanol for later DNA extraction and genetic analysis (see below).

Lizards were returned to the terrestrial ecology facilities at the University of Tasmania, Australia, where they were housed in a temperature- and light-controlled room. The room was set to ambient day length of 12 hours (Hobart, Tasmania, Australia) and each lizard was provided with a basking lamp (set on a timer to come on 1 hour after room lights and turned off 1 hour before rooms lights went off). All lizards were individually housed in identical rectangular plastic terraria (30 x 60 x 40 cm) and provided reptile bedding, a basking rock and the basking lamp at the one end, and a shelter at the opposite end of the terraria. Lizards were provided food (*Tenebrio* larvae) and water *ad libitum*.

We caught lizards prior to hibernation. As the mating season begins soon after emergence from hibernation this ensured that no individuals were paired, or had a mate, prior to the mate discrimination trials. This required us to hibernate lizards for the duration of winter in the laboratory. Following a 2 week settlement period, we began shortening the ambient day length by one hour each week (30 minutes in the morning and 30 minutes in the afternoon), which roughly corresponds with natural conditions (Hobart, Tasmania, Australia). Basking lights were also reduced by the corresponding time. After 6 weeks (once day length had reached a length of 6 hours per week) we switched basking lights off and maintained lizards under ambient day light only for a week before switching all room lights off. Individuals were then kept under these conditions throughout the duration of winter (mid-May to late-July). During this time, the laboratory temperature was maintained at 5^{o}C (corresponding with average temperatures in winter in Tasmania). To maintain a low metabolic rate during hibernation lizards were only provided with water (no light source). In mid-

July, overhead lights were switched back on for 6 hours a day. After a week, basking lights were also switched on to the corresponding day length and food was provided as above. Ambient and basking light day length was then increased by 2 hours per day until ambient day length reached 12 hours per day.

Experimental Design and Data collection

Experimental trials were carried out in late August, which corresponds to the beginning of the spring mating season for this species (GMW pers. obs.). Receptivity of lizards was confirmed by allowing a number of individuals (n=10) to mate following completion of the mate discrimination trials. In all cases mating behaviour, including tongue flicks, chasing and mounting, was observed.

To test discrimination between mates we used Y-mazes. These were constructed from opaque Polyvinyl chloride sheets and cut to 90 cm in length, with a main arm of 45 cm and two arms of 45 cm each and covered with fitted clear perspex lids. Both arms and the main chamber were 10 cm wide. A fan was attached at the end of the main chamber which drew air from the two arms, facilitating the distribution of scent, from each arm, throughout the maze. Partitions were placed at the end of each arm to prevent the focal lizard seeing and coming into physical contact with either potential mate. A 1 cm space underneath each partition allowed air flow from the potential mates to the focal animal, facilitating olfactory communication.

Prior to the experimental treatments, individuals and potential mates were kept in separate temporary plastic terraria. All experiments were run during the day in a temperature controlled room set at 18°C. Two basking lamps (40 W globes) were placed above either end of the Y-maze to provide heat to the subject lizards. The base of the maze was lined with reptile bedding and paper was attached at a height of 3 cm along the walls of the Y-maze to prevent individuals from responding to their reflection.

We ran 21 replicates for each sex separately (yielding 42 replicates in total). Each individual was presented with a choice of two potential mates, which were allocated to one of the two arms of the Y-maze, with one arm containing a closely related mate and the other a less related mate. The arm of the maze containing the

related and unrelated potential mate was randomized for each test. Potential mates were assigned from a spectrum of pairwise relatedness values estimated for all individuals based on prior genetic analysis (see below). The average relatedness of individuals designated as closely-related was 0.16 ± 0.04 and 0.20 ± 0.02 for males and females respectively (all data reported as means ± 1SE), and for individuals designated as less-related was -0.18 ± 0.03 and -0.14 ± 0.02 for males and females respectively (see Table 1 for full range of relatedness values between treatments). This range of relatedness values closely corresponds to the range of mates available in the wild (-0.22 to 0.41 for social and extra-pair mates; see While et al. 2014). Furthermore, the level of relatedness for the more closely-related individual (~0.20) is in accordance with much of the theory that suggests that optimal inbreeding is associated with slightly more distantly related kin as opposed to immediate relatives (Bateson 1982, 1983). The mean difference between related and unrelated potential mates was 0.31 ± 0.04 for males and 0.33 ± 0.03 for females. The closely related and less related potential mates were similar in body size (males; closely related: 79.32 ± 0.72 mm *vs.* less related: 80.32 ± 0.81 mm, females; closely related: 76.26 ± 1.06 mm *vs.* less related: 77.82 ± 1.18; overall effect of treatment across the sexes: $F_{1,87} = 1.79$, $P = 0.18$) and in their capture distance from the focal individuals (closely-related = 83.22 ± 6.95 m *vs.* less-related = 83.71 ± 6.41 m; $F_{1,87} = 0.27$, $P = 0.96$). The latter result allowed us to separate out mate choice based on relatedness *vs.* that based on familiarity.

Table 1: Estimates of the mean of and range in relatedness between focal and the two subject (related and unrelated) individuals for both males and females

	More Related			Less related		
	Mean ± SE	Min	Max	Mean ± SE	Min	Max
Male	0.16 ± 0.04	-0.11	0.68	-0.18 ± 0.03	-0.27	-0.02
Female	0.20 ± 0.02	-0.03	0.37	-0.14 ± 0.02	-0.25	0.06

Focal individuals were placed at the base of the main chamber of the Y-maze, behind a partition, and allowed to acclimatize to the maze for 5 minutes. The partition was then lifted, and the individual released into the Y-maze for 30 minutes. During each trial a number of behaviours were recorded by a single observer (GB). The experimenter was not blind to the experimental replicates, but as we had potential predictions for responses in both directions this should not have entered any bias into the results. First, we recorded the number of tongue flicks the focal individual performed in the main chamber and in each arm. Tongue flicks are a common means of determining the extent of discrimination and preference in lizard studies (e.g. Pernatta et al. 2009; Hews et al. 2011; Lopez and Martin 2012; Scott et al. 2015; Heathcote et al. 2016). However, as there are a number of problems associated with functionally interpreting differential tongue-flicking rates (see Heathcote et al. 2014), we use tongue flick rates here as evidence for discrimination rather than mate choice or preference. Second we recorded the latency it took the focal individual to enter each arm of the maze (in seconds). Finally, we recorded the total amount of time the focal individual spent associated with either stimulus individual in a given arm (in seconds). Social associations between males and females are commonly used as a means to study potential mating decisions in lizards (see Martín and López 2000; López et al. 2002; López and Martín 2005). The experimenter was not blind to the experimental replicates, but as we had predictions for responses in both directions (see introduction) this should have limited to potential to enter bias into the results.

At the completion of each treatment, both the focal individual and the potential mates were removed from the maze, the Y-maze was cleaned with 70% ethanol and dried. Unsuccessful (n = 6) trials occurred when the focal individual remained in the start area for 20 minutes; re-run replicates were conducted the following day with the same focal and subject individuals. At the completion of the experiment all lizards were returned to their site of capture.

Molecular and relatedness analysis

Microsatellite genotyping

DNA was extracted from tail tip samples using the hexadecyltrimethyl ammonium bromide (CTAB) protocol. All individuals used in this study were genotyped for four tetranucleotide microsatellite loci (EST1, EST2, EST4, EST12; Gardner et al. 1999) and two di-nucleotide microsatellite loci (Trl12, Trl 28; Gardner et al. 2008). These loci are highly variable and informative and conform to the expectations of Hardy–Weinberg equilibrium (this study; see also While et al. 2009a; Chapple and Keogh 2005). Results were analysed with Beckman Coulter CEQ8000 Genetic Analysis software.

Estimates of Genetic Relatedness

The program COANSTRY (Wang 2011) was used to estimate pairwise relatedness between males and females. This program calculates the Queller and Goodnight (1989) index of relatedness (R). Standard errors of R estimates were obtained by jack-knifing over the six loci. A single relatedness analysis was performed on all individuals to calculate estimates of pairwise relatedness between all males and females. From this we assigned two potential mates for each individual, one closely related and one distantly related to the focal individual. Potential mates were assigned from a spectrum of pairwise relatedness values estimated for all individuals (Table 1).

Statistical Analysis

For count variables (number of tongue flicks) and binary responses (choice of treatment arm) we ran generalized linear models fit with Poisson and binomial error distributions in the 'lme4' package (Bates et al. 2015), respectively. For time spent in each treatment arm we ran a beta regression using the 'betareg' package (Grün et al. 2012), taking the proportion of total trial time spent in each arm as the response variable. We began by including the main effects of sex and treatment and their pairwise interaction as predictor variables in all models. If interactions terms were non-significant ($P > 0.05$) we dropped them from the model and results from reduced models are reported. In total we ran five separate models with the response variables;

(i) number of tongue flicks in the main chamber; (ii) number of tongue flicks in the treatment arms; (iii) latency to choose one of the treatment arms; (iv) choice of the first arm; and (v) time associated with the each potential mate in the treatment arms (see Table 1 for full model specification). For models in which multiple responses were included for the same focal individual (i.e. count of tongue flicks in each of the two treatment arms) we included a random effect of ID to account for repeated measures. For model (i) and (iii) we included the difference in relatedness between the two potential mates as a covariate in the models to control for variation in the degree of relatedness between potential mates. For our analysis of tongue flicks, we did not control for differences between individuals in the amount of time they spent in the main chamber or the arms because initial inspection of the data showed that there was no relationship between time spent and number of tongue flicks (main chamber: $F_{1,88} = 0.02$, P = 0.88; treatment arms: $F_{1,51} = 0.42$, P = 0.52). For the examination of the number of tongue flicks in the treatment arms we removed all responses in which subjects never entered the arm (i.e. in which time in the treatment arm = 0s). All statistical analyses were conducted using R version 3.3.0 (R Core Team 2013).

Results

The difference in relatedness between the two stimulus animals did not significantly influence either the latency to enter an arm or the number of tongue flicks while in the main chamber for either sex (Table 2). Females performed more tongue flicks than males while in the main chamber before making a choice (Table 2; Fig. 1). Both sexes performed more tongue flicks in the treatment arm containing the less related potential mate (Table 2; Fig. 2).

Both sexes were more likely to choose the arm containing the more-closely related potential mate as their first choice (Table 2). A marginally non-significant treatment-by-sex interaction indicated a trend for males to choose the related arm first more often than females ($p = 0.07$; Table 2). The proportion of time spent in each treatment arm showed the same pattern (Table 2; Fig. 3); the model reported a main effect of treatment, indicating a greater proportion of time spent in the arm containing the more closely-related potential mate with a marginally non-significant treatment by sex interaction.

Table 2: The influence of sex, treatment and the difference in relatedness between potential stimulus choices on discrimination and time association behaviours. Results for main effects are reported from reduced models after non-significant interaction terms were dropped (significance of these terms shown). Dashes represent terms that were not included in the specified model. * indicates that sex was unable to be measured as a main effect in this model because every individual of both sexes made a first choice

Variable	Sex	Treatment	Difference in relatedness	Sex x Treatment
Tongue flicks (main chamber)	**χ^2 = 7.06, P < 0.01**	-	χ^2 = 1.24, P = 0.27	-
Latency	χ^2 = 0.02, P = 0.89	-	χ^2 = 1.05, P = 0.31	-
Tongue flicks (treatment arm)	χ^2 = 0.08, P = 0.77	**χ^2 = 4.54, P = 0.03**	-	χ^2 < 0.01, P = 0.96
First choice	*	**χ^2 = 8.97, P < 0.01**	-	χ^2 = 3.35, P = 0.07
Time in arm	χ^2 = 0.147, P = 0.70	**χ^2 = 5.91, P = 0.02**	-	χ^2 = 3.27, P = 0.07

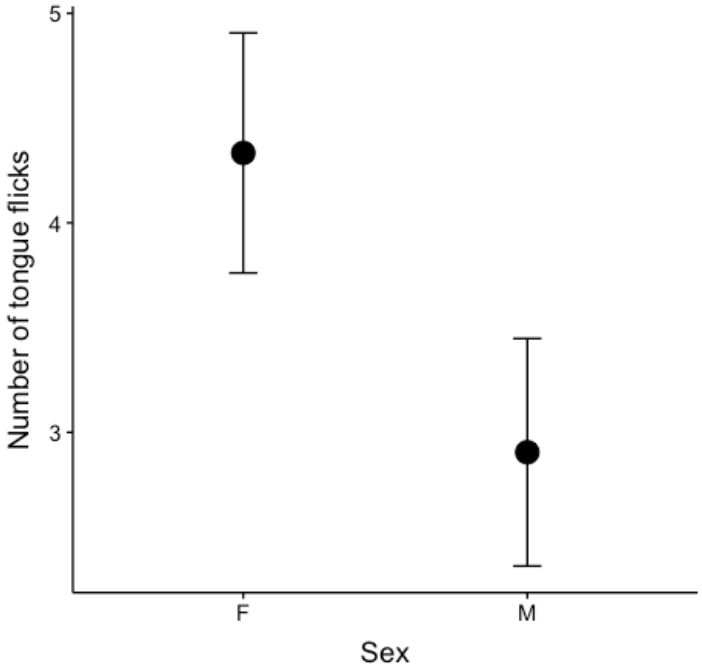

Fig. 1: Mean (±SE) number of tongue flicks performed by males and females in the main chamber of the Y-maze.

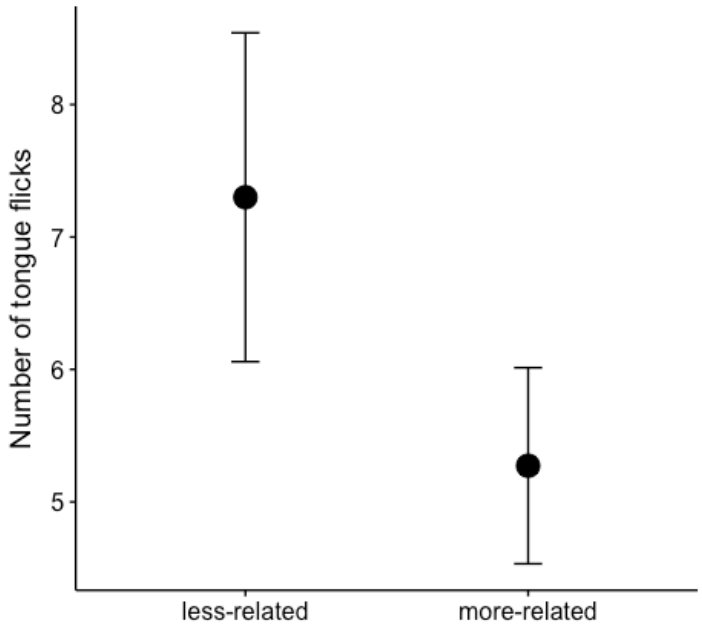

Fig. 2 Mean (±SE) number of tongue flicks performed in the less-related and more-related treatment arm of the Y-maze for males and females combined

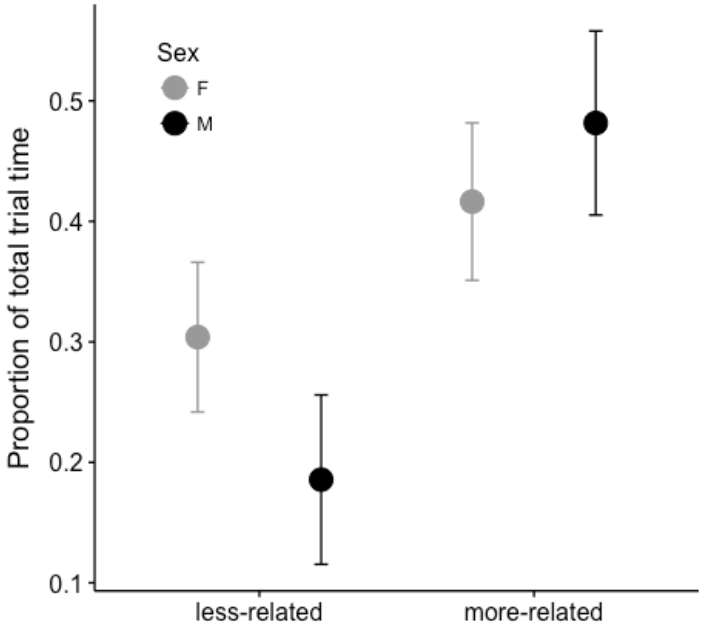

Fig. 3 Proportion (±SE) of total trial time spent in the less-related and more-related treatment arm for males and females

Discussion

Previous research has identified non-random mating patterns of mate choice in this population of *L. whitii*. Males and females pair with closely related individuals to a greater extent than expected by chance (While et al. 2014). This has been suggested to be a consequence of limited natal dispersal resulting in substantial genetic structuring which, in combination with low breeder turn over, constrains social mate encounter rates and thus biases pairing towards relatives (While et al. 2014). However, recent theoretical, and growing empirical, research has cautioned against such interpretations and has, instead, suggested that such patterns may be the result of active preference for closely related individuals (Kokko and Ots 2006; Oh 2011). Here, we confirm that male and female *L. whitii* distinguish between individuals based on their relatedness and, furthermore, both sexes preferentially associate with more closely related members of the opposite sex.

Tongue flicks are commonly used to measure discrimination in squamate reptiles (Pernatta et al. 2009; Hews et al. 2011; Lopez and Martin 2012; Scott et al. 2015; Heathcote et al. 2016) as olfactory communication is the primary means of social communication in this taxon. Consistent with our prediction, both sexes showed a difference in the number of tongue flicks directed towards related *vs.* unrelated potential mates. Specifically, individuals tongue flicked more when in the arm with a less related potential mate compared to the arm with a more related potential mate. This result suggests an ability to discriminate between potential mates based on their genetic similarity, which is consistent with research on a number of other species in the *Egernia* group (e.g. Main and Bull 1996; Bull et al. 2001; O'Connor and Shine 2006). In further support of this, we also found that individuals biased their choice of arms, both in terms of their initial preference and the amount of time spent in the arms. Given that we restricted all other modes of discrimination, these results strongly suggest an ability to discriminate between related and unrelated individuals based on scent in *L. whitii* (see also While et al. 2009b, 2014).

The strong difference in the amount of time spent associated with related *vs.* unrelated potential mate, when combined with patterns of mate choice from the natural population (While et al. 2014), suggests that mate choice with respect to

relatedness may be an active rather than passive process in this population. This interpretation is consistent with a growing body of literature demonstrating a positive preference for pairing with kin across a range of taxa (e.g. Kleven et al. 2005; Thünken et al. 2007; Langen et al. 2011; Wang and Lu 2011; Robinson et al. 2012). Inbreeding preference is predicted to emerge whenever the inclusive fitness benefits outweigh the costs of reduced offspring quality as a result of inbreeding depression (Waser et al. 1998; Parker 2006; Szulkin et al. 2013). Research from wild populations of birds have begun to show that individuals can gain substantial inclusive fitness benefits from inbreeding, despite reduced offspring quality (Reid et al. 2015). To fully understand the extent to which mate choice in *L. whitii* is a result of passive constraints associated with population viscosity or an active preference for mating with related individuals, similar long-term data on the consequences of inbreeding and outbreeding for both males and females are required. Such an approach also has the potential to shed light on the heritability of inbreeding preference, and thus the degree to which inbreeding systems might evolve in response to selection (see Wolak and Reid 2016). It may also allow us to examine the extent to which the benefits of inbreeding differ between within-pair and extra-pair copulations. Theoretical models have suggested that optimal inbreeding strategies can differ substantially between mating systems, both within and among populations (Lehtonen and Kokko 2015). In our natural population, individuals pair with closely related individuals but pursue/accept extra-pair copulations from less related individuals (While et al. 2014). If the patterns of social mate choice observed in our natural population are the result of a preference for closely related individuals, we require an explanation for why individuals choose less related individuals as extra-pair partners. Speculation on this topic is outside the scope of this paper but provides an exciting potential direction for future research.

We also found that the extent of preference for related individuals differed, to some extent, between the sexes. Specifically, males tended to exhibit a stronger preference for more closely related partners than did females both in terms of initial choice and time spent in association. While these differences did not reach statistical significance, potentially due to limited sample size, they are suggestive of potential sexual conflict over inbreeding. Producing inbred offspring is expected to have

increased fitness consequences for females compared to males due to unequal patterns of reproductive investment (Pizzari et al. 2004; Parker 2006; Duthie and Reid 2015). Therefore, females should exhibit a greater plasticity in their mate preferences in response to social context compared to males, potentially resulting in weaker overall effect sizes for inbreeding preference.

One caveat to our study is that our experimental design relies on the assumption that our association data represent a mating preference (see methods for studies that have used this approach in the past). An alternative interpretation is that these results represent a more general preference for more closely related individuals. This could be to avoid aggressive behaviour as relatedness can moderate aggression between individuals (Carazo et al. 2014; Griesser et al. 2015) and previous research on mate choice in lizards has shown a preference for less threatening males in the laboratory (opposite to the patterns observed in the wild; Carazo et al. 2011) or because clusters of closely related individuals represent a cue to that individual's home range. While these possibilities cannot be ruled out without additional experimental trials (e.g. examining the extent to which aggression between individuals co-varies with relatedness, intra *vs.* inter sexual preference trials, staged mating trials), low levels of inter-sexual aggression compared to intra-sexual aggression observed in both natural and experimental conditions (While et al. 2009a; Halliwell et al. 2016) combined with the consistency of preference observed in the wild and the laboratory suggest these interpretations are unlikely. Alternatively, a preference for kin may be explained by a broader function for kin recognition as a result of parental care/tolerance which is important in this species (e.g. While et al. 2009b). Nonetheless, regardless of the evolutionary origin of a mechanism for kin recognition in this system, this could be co-opted to serve to enable distinguish between related and unrelated individuals in a mating context, as suggested by our previous work (While et al. 2014; this study).

In summary, our results suggest that mate choice for related individuals within our system is the result of an active preference for related individuals, rather than a constraint on optimal outbreeding as a result of population viscosity. However, in order to understand the extent to which such preferences for inbreeding are adaptive, we must integrate these findings with the long-term fitness costs and benefits of

inbreeding/outbreeding (see Reid et al. 2015). At a taxonomic scale, these data can be combined with patterns of mate choice with respect to inbreeding observed across the broader *Egernia* group (within which *L. whitii* resides). The *Egernia* group consists of species which vary in key ecological, social (varying from solitary to large communal family groups) and life-history characteristics (see Chapple 2003; While et al. 2015) that have the potential to influence the trade-off between the inclusive fitness benefits and the costs of inbreeding. These species also exhibit different inbreeding strategies at both the social and extra-pair level (with inbreeding avoidance, tolerance and preference observed; Bull and Cooper 1999; Gardner et al. 2001; Stow and Sunnocks 2004; Chapple and Keogh 2005). This has the potential to provide an outstanding comparative framework in which we can explore the ecological and life history conditions under which animals make mating decisions related to inbreeding. Such an approach will allow us to move away from asking whether a population exhibits inbreeding avoidance or tolerance and towards a more holistic approach which focuses on the context dependent nature of the costs and benefits of inbreeding. Ultimately this will enable us to identifying the ecological, social and life-history traits which are likely to predict when individuals should avoid, tolerate or prefer relatives as mates (Szulkin et al. 2013).

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References

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw doi:10.18637/jss.v067.i01

Bateson PPG (1982) Preferences for cousins in Japanese quail. Nature 295:236–237

Bateson P (1983) Optimal outbreeding. In: Bateson P (ed) Mate choice. Cambridge University Press, Cambridge, pp 257-277

Bretman A, Newcombe D, Tregenza T (2009) Promiscuous females avoid inbreeding by controlling sperm storage. Mol Ecol 18:3340–3345

Bull CM, Cooper SJB (1999) Relatedness and the avoidance of inbreeding in the lizard, Tiliqua rugosa. Behav Ecol Sociobiol 46:367–372

Bull CM, Griffin CL, Bonnett M, Gardner MG, Cooper SJB (2001) Discrimination between related and unrelated individuals in the Australian lizard *Egernia striolata*. Behav Ecol Sociobiol 50:173–179

Carazo P, Font E, Desfilis E (2011) The role of scent marks in female choice of territories and refuges in a lizard *Podarcis hispanica*. J Comp Psychol 125:362–365

Carazo P, Tan CK, Allen F, Wigby S, Pizzari T (2014) Within-group male relatedness reduces harm to females in *Drosophila*. Nature 505:672–675

Chapple DG (2003) Ecology, life-history, and behavior in the Australian Scincid genus *Egernia*, with comments on the evolution of complex sociality in lizards. Herpetol Monogr 17:145–180

Chapple DG, Keogh JS (2005) Complex mating system and dispersal patterns in a social lizard, *Egernia whitii*. Mol Ecol 14:1215–1227

Charlesworth D (2006) Evolution of plant breeding systems. Curr Biol 16:726–735

Charlesworth D, Willis JH (2009) The genetics of inbreeding depression. Nat Rev Genet 10:783–796

Duthie AB, Reid JM (2015) What happens after inbreeding avoidance? Inbreeding by rejected relatives and the inclusive fitness benefit of inbreeding avoidance. PLoS ONE 10:e0125140

Fitzpatrick JL, Evans JP (2014) Postcopulatory inbreeding avoidance in guppies. J Evol Biol 27:2686–2694

Fitzpatrick LJ, Casparinin C, Fitzpatrick JL, Evans JP (2014) Male-female relatedness and patterns of male reproductive investment in guppies. Biol Lett 10:20140166

Gardner MG, Cooper SJB, Bull CM, Grant WN (1999) Isolation of microsatellite loci from a social lizard, *Egernia stokesii*, using a modified enrichment procedure. J Hered 90:301– 304

Gardner MG, Bull CM, Cooper SJB, Duffield GA (2001) Genetic evidence for a family structure in stable social aggregations of the Australian lizard *Egernia stokesii*. Mol Ecol 10:175–183

Gardner MG, Sanchez JJ, Dudaniec RY, Rheinberger L, Smith AL, Saint KM (2008) *Tiliqua rugosa* microsatellites: isolation via enrichment and characterisation of loci

for multiplex PCR in *T. rugosa* and the endangered *T. adelaidensis*. Conserv Genet 9:233–237

Griesser M, Halvarsson P, Drobniak SM, Vila C (2015) Fine-scale kin recognition in the absence of social familiarity in the Siberian jay, a monogamous bird species. Mol Ecol 24:5726–5738

Grün B, Kosmidis I, Zeileis A (2012). Extended beta regression in R: shaken, stirred, mixed, and partitioned. J Stat Softw doi:10.18637/jss.v048.i11

Halliwell B, Uller T, Wapstra E, While GM (2016) Resource distribution mediates social and mating behavior in a family living lizard. Behav Ecol (published online, doi:10.1093/beheco/arw134)

Heathcote RJP, Bell E, D'Ettorre P, While GM, Uller T (2014) The scent of sun worship: basking experience alters scent mark composition in male lizards. Behav Ecol Sociobiol 68:861–870

Heathcote RJP, While GM, McGregor HEA, Sciberras J, Leroy C, D'Ettore P, Uller T (2016) Male behaviour drives assortative reproduction during the initial stages of secondary contact. J Evol Biol 29:1003–1015

Hews DK, Date P, Hara E, Castellano MJ (2011) Field presentation of male secretions alters social display in *Sceloporus virgatus* but not *S. undulates* lizards. Behav Ecol Sociobiol 65:1403–1410

Keller LF, Arcese P (1998) No evidence for inbreeding avoidancve in a natural population of song sparrows (*Melospiza melodia*). Am Nat 152:380–392

Keller LF, Waller DM (2002) Inbreeding effects in wild populations. Trends Ecol Evol 17:19–23

Kleven O, Jacobsen F, Robertson RJ, Lifjeld JT (2005) Extrapair mating between relatives in the barn swallow: a role for kin selection? Biol Lett 1:389–392

Kokko H, Ots I (2006) When not to avoid inbreeding. Evolution 60: 467–475

Langen K, Schwarzer J, Kullmann H, Bakker TCM, Thünken T (2011) Microsatellite support for active inbreeding in a cichlid fish. PLoS ONE 6:e24689

Lehtonen J, Kokko H (2015) Why inclusive fitness can make it adaptive to produce less fit extra-pair offspring. Proc R Soc B 282:20142716

Liu X, Ty X, He H, Chen C, Xue F (2014) Evidence for inbreeding depression and pre-copulatory but not post-copulatory inbreeding avoidance in the cabbage beetle *Colaphellus bowringi*. PLoS ONE 9:e94389

López P, Martín J (2005) Female Iberian wall lizards prefer male scents that signal a better cell-mediated immune response. Biol Lett 1:404–406

López P, Martín J (2012) Chemosensory exploration of male scent by female rock lizards result from multiple chemical signals of males. Chem Senses 37:47–54

López P, Muñoz A, Martín J (2002) Symmetry, male dominance and female mate preferences in the Iberian rock lizard, *Lacerta monticola*. Behav Ecol Sociobiol 52:342–347

Main AR, Bull CM (1996) Mother-offspring recognition in two Australian lizards, *Tiliqua rugosa* and *Egernia stokesii*. Anim Behav 52:193–200

Martín J, López P (2000) Chemoreception, symmetry and mate choice in lizards. Proc R Soc Lond B 267:1265–1269

O'Connor DE, Shine R (2006) Kin discrimination in the social lizard *Egernia saxatilis* (Scincidae). Behav Ecol 17:206–211

Oh KP (2011) Inclusive fitness of 'kissing cousins': new evidence of a role for kin selection in the evolution of extra-pair mating in birds. Mol Ecol 20:2657–2659

Olson LE, Blumstein DT, Pollinger JR, Wayne RK (2012) No evidence for inbreeding avoidance despite demonstrated survival costs in a polygynous rodent. Mol Ecol 21:562–571

Parker GA (1979) Sexual selection and sexual conflict. In: Blum MS, Blum NA (eds) Sexual selection and reproductive competition in insects. Academic Press, New York, pp 123-166

Pernatta AP, Reading CJ, Allen JA (2009) Chemoreception and kin discrimination by neonate smooth snakes, *Coronella austriaca*. Anim Behav 77:363–368

Pizzari T, Lovlie H, Cornwallis CK (2004) Sex-specific counteracting responses to inbreeding in a bird. Proc R Soc Lond B 271:2115–2121

Pusey A, Wolf M (1996) Inbreeding avoidance in animals. Trends Ecol Evol 11:201–206

Puurtinen M (2011) Mate choice for optimal (k)inbreeding. Evolution 65:1501–1505

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43:258–275

R Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org/

Reid JM, Arcese P, Bocedi G, Duthie AB, Wolak ME, Keller LF (2015) Resolving the conundrum of inbreeding depression but no inbreeding avoidance: estimating sex-specific selection on inbreeding by song sparrows (*Melospiza melodia*). Evolution 69:2846–2861

Robinson SP, Kennington WJ, Simmons LW (2012) Assortative mating for relatedness in a large naturally occurring population of *Drosophila melanogaster*. J Evol Biol 25:716–725

Scott M, Llewelyn J, Higgie M, Hoskin C, Pike K, Phillips B (2015) Chemoreception and mating behaviour of a tropical Australian skink. Acta Ethol 18:289–293

Smith RH (1979) On selection for inbreeding in polygynous animals. Heredity 43:205–211

Stow AJ, Sunnocks P (2004) Inbreeding avoidance in Cunningham's skinks (*Egernia cunninghamii*) in natural and fragmented habitat. Mol Ecol 13:443–447

Szulkin M, Stopher KV, Pemberton J, Reid JM (2013) Inbreeding avoidance, tolerance, or preference in animals. Trends Ecol Evol 28:205–211

Thünken T, Bakker TCM, Baldauf SA, Kullmann H (2007) Active inbreeding in a cichlid fish and its adaptive significance. Curr Biol 17:225–229

Wang C, Lu X (2011) Female ground tits prefer relatives as extra-pair partners: driven by sexual selection? Mol Ecol 20:2851–2863

Wang J (2011) COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. Mol Ecol Resour 11:141–145

Waser PM, Austad SN, Keane B (1986) When should animals tolerate inbreeding? Am Nat 128:529–537

While GM, Chapple DG, Gardner MG, Uller T, Whiting MJ (2015). *Egernia* lizards. Curr Biol 25:593–595

While GM, Uller T, Bordogna G, Wapstra E (2014) Promiscuity resolves constraints on social mate choice imposed by population viscosity. Mol Ecol 23:721–732

While GM, Uller T, Wapstra E (2009a) Variation in social strategies characterise the social and mating system of an Australian lizard, *Egernia whitii*. Aust Ecol 34:938–949

While GM, Uller T, Wapstra E (2009b) Family conflict and the evolution of sociality in a non-avian vertebrate. Behav Ecol 20:245–250

While GM, Uller T, Wapstra E (2011) Variation in social organisation influences the opportunity for sexual selection in a social lizard. Mol Ecol 20:844–852

Wolak ME, Reid JM (2016) Is pairing with a relative heritable? Estimating female and male genetic contributions to the degree of biparental inbreeding in song sparrows (*Melospiza melodia*). Am Nat 187:736–752